National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1988-September 30, 1989

U.S. Department of Health and Human Services
Public Health Service
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Annual Report of Intramural Activities

October 1, 1988-September 30, 1989

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1989 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00013-26 LIP
00020-14 OSD
00027-22 LMM
00030-21 LI
00035-14 LI
00036-24 LI
00043-24 LCI
00045-21 LCI
00047-20 LCI
00048-19 LCI
00057-16 LCI
00058-15 LCI
00071-18 LPB
00072-18 LPVD
00074-17 LPVD
00085-12 LPVD
00086-12 LPVD
00094-30 LPD
00097-31 LPD
00098-33 LPD
00099-19 LPD
00102-15 LPD
00108-18 LPD
00123-23 LVD
00126-16 LVD
00134-27 LI
00135-15 LIP
00143-20 OSD
00144-25 OSD
00145-22 OSD
00154-14 LCI
00155-14 LCI
00161-12 LPD
00162-13 LPD
00166-12 LIG
00168-12 LIG
00169-12 BRB
00170-12 LIG
00171-12 LIG
00172-11 BRB
00173-12 LIG
00180-11 LIG
00182-11 OSD
00183-11 OSD
00190-11 LMM
00193-10 LMSF
00197-10 LPD
00201-10 LPB
00205-09 LIP

Z01-AI
00208-09 LPD
00210-09 LIR
00212-09 LIR
00213-09 LIR
00216-09 LMSF
00218-08 LMM
00224-08 LI
00226-08 LI
00240-08 LPD
00241-08 LPD
00244-08 LPD
00246-07 LPD
00248-08 LPD
00249-08 LCI
00251-08 LPD
00253-08 LPD
00255-08 LPD
00256-08 LPD
00257-08 LPD
00260-08 LPVD
00262-08 LPVD
00263-08 LPVD
00264-08 LPVD
00265-08 LPVD
00266-08 LPVD
00271-08 LCI
00275-08 LCI
00278-08 LCI
00279-08 LCI
00284-08 LIP
00286-08 LIP
00294-08 LVD
00295-08 LVD
00296-08 LVD
00297-08 LVD
00298-08 LVD
00300-08 LMM
00301-08 LMM
00304-08 LMM
00306-08 LVD
00307-08 LVD
00309-08 LID
00311-08 LID
00314-08 LID
00317-08 LID
00323-08 LID
00324-08 LID
00325-08 LID
00326-08 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1989 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00327-08 LID
00333-08 LID
00339-08 LID
00340-08 LID
00341-08 LID
00342-08 LID
00343-08 LID
00345-08 LID
00346-08 LID
00347-07 LPD
00349-07 LI
00350-07 LPD
00351-07 LPD
00352-07 BRB
00353-07 LMM
00354-07 LCI
00356-07 LCI
00358-07 LIR
00361-07 LIR
00368-07 LID
00370-07 LID
00372-07 LID
00389-06 LIG
00390-06 LIR
00394-06 LI
00397-06 LCI
00402-06 LPB
00403-06 LI
00406-06 LID
00415-06 LMM
00416-06 LVD
00418-06 LPVD
00425-05 LI
00427-05 LI
00429-05 LCI
00430-05 LCI
00431-05 LIR
00432-05 LCI
00437-05 LMM
00438-05 LMM
00439-05 LPD
00440-05 LID
00441-05 LMSF
00445-05 LVD
00446-05 LID
00450-05 LID
00451-05 LID
00459-05 LID
00465-04 LIP

Z01-AI
00467-04 LMM
00468-04 LPVD
00469-04 LCI
00470-04 LCI
00477-04 LID
00478-04 LID
00480-04 LPB
00481-04 LCI
00483-04 LPD
00484-03 LIP
00485-03 LCMI
00486-03 LCMI
00487-03 LPD
00488-03 LPB
00489-03 LPB
00490-03 LPB
00491-03 LPB
00492-03 LPB
00493-03 LI
00494-03 LPD
00495-03 LCI
00496-03 LID
00497-03 LID
00498-03 LID
00499-03 LID
00500-03 LID
00501-03 LID
00502-03 LID
00507-03 LID
00512-02 LPD
00513-02 LCI
00514-02 LCI
00516-02 LMSF
00517-02 LPD
00518-02 LMSF
00519-02 LMSF
00520-02 LI
00521-02 LCI
00522-02 BRB
00523-02 BRB
00524-02 LPVD
00525-02 LIG
00527-02 LMM
00528-02 LMM
00529-02 LCMI
00530-02 LID
00531-02 LID
00532-02 LID
00533-02 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1989 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00534-02 LID
00535-02 LPD
00536-02 LPD
00537-02 LIR
00538-02 LVD
00539-02 LVD
00540-02 LVD
00541-02 LVD
00542-02 LVD
00543-02 BRB
00544-01 LIP
00545-01 LI
00546-01 LMM
00547-01 LMM
00548-01 LCI
00549-01 LMSF
00550-01 LPVD
00551-01 LPVD
00552-01 LPB
00553-01 LPB
00554-01 LPB
00555-01 LID
00556-01 LID
00557-01 LID
00558-01 LID
00559-01 LID
00560-01 LID
00561-01 LID
00562-01 LID
00563-01 LVD
00564-01 LVD

OSD

OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID
1989 Annual Report
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Summary of Program
Laboratory and Clinical Research NIAID
October 1, 1988 - September 30, 1989

The Division of Intramural Research (DIR), NIAID consists of 13 laboratories, a Biological Resources Branch, Administrative Management Branch and Animal Care Branch. Three laboratories are located in Hamilton, Montana at the Rocky Mountain Laboratories. The remaining facilities are concentrated at the NIH Bethesda Campus with auxiliary facilities at Frederick (Ft. Detrick) and Rockville, MD. The Office of the Director, DIR, (also called the Office of the Scientific Director, NIAID) is responsible for the management of the Division of Intramural Research as well as advising the Director, NIAID, on scientific issues. In FY'89 the DIR, NIAID, had an allocation of $92,052,000 with 25.39% for AIDS research and 74.61% for other than AIDS research. About 31% of the budget was for salaries, 24% for the NIH management fund and the remainder for research support with about 28% for direct operations of laboratories and 17% for contracts to support laboratory operations. In FY '89 there were 811 persons working within DIR, NIAID, with 87 tenured scientists, 530 nontenured scientists (including guest workers and special volunteers) and 194 nonscientists.

This past year there was continued renovation of the NIAID facility in Rockville, MD which included the initiation of construction to modify space to create an animal facility to support NIAID investigators. There was also considerable modernization of existing facilities on the Bethesda campus, with emphasis on NIAID space in the clinical center. The local area network linking all laboratories within NIAID by computer was expanded to include virtually all scientists in the DIR.

Numerous outstanding accomplishments were made by DIR, NIAID scientists and are summarized in this annual report. Some of the many highlights in other than AIDS research were as follows. The genes for two forms of chronic granulomatous disease of childhood were cloned and sequenced and a new plasma protein that interacts with complement and kinin forming pathway that may be an important mediator on the disease hereditary angioedema was identified, cloned and partially sequenced. Clinical trials of interferon gamma in chronic granulomatous disease of childhood and angioedema were initiated. Studies of the inflammation associated with allergic responses showed that cells of the mast cell lineage may be key regulators of allergic responses, not simply through the release of histamine, but also through the recruitment of the cellular inflammatory elements of this system from mast cells, including lymphokines Il-3, Il-4, Il-5 and granulocyte-macrophage colony stimulating factor. In studies to develop a safe whooping cough vaccine a genetically inactivated pertussis toxin, which may be the basis for new-generation whooping cough vaccines, was developed. Acyclovir was proven ineffective in treating the symptoms of chronic fatigue syndrome and 75% of patients with chronic fatigue syndrome were found to have a history of psychiatric illness. RML scientists have developed a sensitive, specific assay of the Lyme disease spirochete, Borrelia burgdorferi, using the polymerase chain reaction, and in other studies, RML scientists provided evidence that Borrelia burgdorferi is highly susceptible to genetic alterations rendering it resistant to naturally occurring antibodies. The basis for chronic inflammation and scarring of the fallopian tube and ocular sites following infection with chlamydial was linked to inflammatory damage from hypersensitivity to a 57-kDa chlamydial protein. Several host genes have been identified which are involved in resistance to induction of degenerative brain disease by the scrapie agent and altered cellular proteins have been found in mouse brain tumor cells infected in vitro with the scrapie agent.

Special highlights of research related to AIDS include the following. It was shown that an HIV Nef protein slows viral production. A soluble CD4 linked to toxin showed potential as a new AIDS treatment. Based in part on studies by DIR NIAID scientists, alpha interferon was approved by the FDA for treatment of AIDS-associated Kaposi's sarcoma. In addition alpha interferon administered to persons infected with HIV, but with no symptoms of AIDS, was shown to slow virus production and reduce the risk of developing AIDS-related opportunistic infections. Studies with simian immunodeficiency virus from sooty mangabey monkeys provided new evidence that this virus may have successfully infected humans 20 to 40 years ago and evolved into HIV-2. Progress in animal models of AIDS continued with DIR NIAID scientists expanding their work on the transgenic mouse, rabbit and feline models of AIDS.

Equal Employment Opportunity and Affirmative Action Programs have been given a high level of attention this year. Our annual Introduction to Biomedical Research Program for Minority Students from across the nation was held in February and continues to be a big success. In an effort to enhance relations between the DIR, NIAID, and minority medical colleges, Deans of several minority medical colleges were invited to attend informal sessions with the Director, DIR, NIAID, and provide advice on new directions for recruitment of minority scientists to NIAID. The Director, DIR, NIAID, has also initiated visits to minority medical schools. As a result of these efforts, a new sabbatical program for minority scientists to work in NIAID laboratories has been initiated. This year NIAID continued its minority summer program for high school, college and medical students with 50 students working in NIAID laboratories; 50% of students in the summer program were minorities.

May 22-24, 1989 the NIAID Board of Scientific Counselors, under the leadership of Dr. Robert B. Couch, reviewed the NIAID Rocky Mountain Laboratories in Hamilton, Montana. The Laboratory of Pathobiology (Dr. Claude F. Garon, Laboratory Chief), the Laboratory of Persistent Viral Diseases (Dr. Bruce Chesebro, Laboratory Chief) and the Laboratory of Microbial Structure and Function (Dr. John Swanson, Laboratory Chief) were reviewed. Each of these laboratories received laudatory reviews and the Rocky Mountain facility was felt to provide an excellent research environment. This year Dr. Robert B. Couch completed his tenure on the Board. Dr. Couch's advice as Chairman of the Board was deemed extremely valuable by the Director, DIR, NIAID, and Dr. Couch was thanked for his years of service to DIR, NIAID.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00143-20 OSD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Genetic Control of the Antibody Response to Microbial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	P. J. Baker	OSD, NIAID	Head, MI Section
Others:	P. W. Stashak	OSD, NIAID	Microbiologist
	M. B. Fauntleroy	OSD, NIAID	Biologist
	K. L. Holmes	BRB, NIAID	Senior Investigator
	J. R. Hiernaux*		
	E. A. Goidl**		
	S. J. M. McEvoy**		

COOPERATING UNITS *(if any)* *Ribi ImmunoChem Research, Inc., Post Office Box 1409, Hamilton, MT 59840; **Department of Microbiology and Immunology, University of Maryland, School of Medicine, Baltimore, MD 21201

LAB/BRANCH

Office of the Director, Intramural Research Program

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION NIAID, NIH, Twinbrook II Research Facility, 12441 Parklawn Drive, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	0.1	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

RIIIS/J mice lack an autosomal gene(s) that influences the magnitude of the antibody response to a wide variety of microbial polysaccharide antigens of biomedical importance. Although the precise nature of this genetic defect remains to be defined, RIIIS/J mice provide an experimental model that can be used to elucidate genetic mechanisms involved in the development of protective immunity and host resistance to disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00144-25 OSD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. J. Baker	OSD, NIAID	Head, MI Section
Others:	P. W. Stashak	OSD, NIAID	Microbiologist
	M. B. Fauntleroy	OSD, NIAID	Biologist
	J. R. Hiernaux*		
	J. L. Cantrell*		
	J. A. Rudbach*		

COOPERATING UNITS *(if any)* *Ribi ImmunoChem Research, Inc., Post Office Box 1409, Hamilton, MT 59840

LAB/BRANCH

Office of the Director, Intramural Research Program

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION NIAID, NIH, Twinbrook II Research Facility, 12441 Parklawn Drive, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	0.6	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ [✓ (c) Neither]
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Treatment with both monophosphoryl lipid A (MPL) and trehalose dimycolate (TDM) cause a significant increase in the magnitude of the antibody response to Type III pneumococcal polysaccharide (SSS-III). The additive nature of the effects produced suggests that these immunomodulators act by different mechanisms; MPL eliminates the inhibitory effects produced by suppressor T cells, whereas TDM causes antigen-stimulated B cells to proliferate further. MPL augments the antibody response of lipopolysaccharide-responder C3H/HeSnJ mice — but not lipopolysaccharide non-responder C3H/HeJ mice — to SSS-/III. Since both strains of mice express the same degree of suppressor T cell activity upon exposure to SSS-III, these findings imply that the suppressor T cells of C3H/HeJ mice are refractory to inactivation by MPL, perhaps because they lack a cell surface receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00145-22 OSD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. J. Baker	OSD, NIAID	Head, MI Section
Others:	M. B. Fauntleroy	OSD, NIAID	Biologist
	P. W. Stashak	OSD, NIAID	Microbiologist
	J. R. Hiernaux*		
	J. L. Cantrell*		
	J. A. Rudbach*		

COOPERATING UNITS *(if any)* * Ribi ImmunoChem Research, Inc., Post Office Box 1409, Hamilton, MT 59840

LAB/BRANCH

Office of the Director, Intramural Research Program

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION NIAID, NIH, Twinbrook II Research Facility, 12441 Parklawn Drive, Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
	0.3	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Treatment with non-toxic monophosphoryl lipid A (MPL), derived from a Re-mutant of *Salmonella typhimurium*, was found to inactivate suppressor T cell activity without altering the expression of amplifier, helper, and delayed type hypersensitivity activity of T cells. Treatment with MPL not only increased the magnitude of the IgM antibody response to Type III pneumococcal polysaccharide (SSS-III) in young mice, but also resulted in the appearance of significant numbers of cells making IgG_1 and IgG_3 antibody specific for SSS-III. These findings indicate that MPL can be used as an adjuvant to improve the immunogenicity of poorly immunogenic polysaccharide vaccines in young, immunologically immature individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-14 OSD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Treatment of Disease with the Interferon System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. B. Levy	Special Asst. to the Dir.	OSD, NIAID

COOPERATING UNITS *(if any)* Dr. A. Salazar, Walter Reed Army Hospital; Dr. J. Morales, Presbyterian Hospital; Dr. S. Baron, University of Texas Medical School, Galveston, TX; Virology Division and Disease Control Division, USAMRIID

LAB/BRANCH

Office of the Director, Division of Intramural Research

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) PolyICLC, with or without zidovudine, has been given to 17 very advanced AIDS patients as the first part of a phase I study. The drug was well tolerated with very slight side effects. Patients reported a weight gain, stabilization of T4 counts in most. After four months, in 6/9 patients, no HIV was detectable; however, these results are too preliminary to be considered significant.

In previous studies, we have found that when polyICLC was given i.v. to patients with M.S., there was improvement, but the side effects were very severe. Dr. Salazar found that when the drug was given i.m to M.S. patients, the adverse reactions were very mild. While it is difficult to evaluate, the i.m.-treated patients appeared to stabilize while on treatment to a degree comparable to the i.v.-treated group.

The USAMRIID, through contracts with several universities, compared the efficacy of a number of antivirals. The evaluating committee plans to do a study with polyICLC in human volunteers infected with Sandfly Fever Virus.

2) A database file was established to keep track of the NIAID employee serum repository. The sampling procedures were reorganized.

3) A computer-based program was developed for the animal research protocols that enable the ACUC to be in compliance with the requirements of the USDA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00182-11 OSD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. C. Williams, Ph.D., Sr. Scietnist, NIAID/OSD, Chief Department of Intracellular Pathogens/Bacteriology Division, USAMRIID
T. A. Hoover, Ph.D., USAMRIID
N. Bhatnagar, Ph.D., National Research Council Fellow.
S-Y. Chen, M.S., Pre-doctoral candidate

COOPERATING UNITS *(if any)*

M. H. Vodkin, Ph.D., University of Illinois, Urbanna, IL
H. A. Thompson, Ph.D., Visiting Scientist and Associate Professor, Dept. Microbiol and Immunol, West Virginia University Medical Center, Morgantown, WV

LAB/BRANCH

Office of the Director of Intramural Research Program, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD and Bldg. 550, Ft. Detrick, Frederick, MD 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project in this reporting period were i) the cloning and sequencing of the origin of replication of the Coxiella burnetii chromosome and ii) the initiation of the mapping of the chromosome. A. Characterization of the DNA replication origin of the C. burnetii chromosome. The origin of chromosome replication of C. burnetii has been cloned and sequenced. The smallest fragment containing the oriC function is 448 base pairs. The origin is unique to C. burnetii with low homology to the oriC of Escherichia coli and other gram negative bacteria. B. Mapping the C. burnetii genome. The generation of a restriction site map was accomplished using eight base cutter restriction enzymes (NotI and SfiI) and pulsed field gel electrophoresis. Selected DNA probes were able to identify bands for future ordering of the map. C. Significance. With the information gathered by these studies we will be able to map the C. burnetii genome. This will facilitate the assignment of virulence markers to certain segments of the chromosome. The origin of replication will be used to prepare mini-chromosomes in the construction of vectors for the movement of DNA in-and-out of C. burnetii. The use of avirulent strains of C. burnetii as a vaccine vehicle will provide a heat stable, live attenuated vehicle for the delivery of selected antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00183-11 OSD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Properties of Coxiella burnetii (Q fever) Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Chief Department of Intracellular Pathogens/Bacteriology Division, USAMRIID
G. H. Scott, Ph.D., USAMRIID
D. W. Waag, Ph.D., USAMRIID
C. R. Bolt, M.S., USAMRIID
T. F. McCaul, Ph.D., Sr. Fellow, National Research Council Fellow
T. A. Hoover, Ph.D., USAMRIID
N. Bhatnagar, Ph.D., National Research Council Fellow

COOPERATING UNITS *(if any)*

H. A. Thompson, Ph.D., Visiting Scientist and Associate Professor, Dept Microbiol & Immunol, West Virginia University Medical Center, Morgantown, WV

LAB/BRANCH

Office of the Director of Intramural Research Program, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project in this reporting period were i) the ultrastructural and biochemical characterization of the morphological cell types of Coxiella burnetii, ii) the identification of cell types undergoing antigenic changes during growth, iii) the immunoblot conditions for the detection of antigens, and iv) the inactivation of C. burnetii with disinfectants. A. Localization of DNA in the spore of C. burnetii. DNA was shown to segregate into the spore and the mother cell during the process of sporulation by C. burnetii. The sporogenic differentiation process consisted of the synthesis of primary and secondary septa which effectively circumscribed both cytoplasm and DNA to form an endogenous spore. The sacrificed mother cell released the spore. B. Antigenic structure of the cell types. A small cell variant-resting cell was identified as a pressure resistant dormant cell without a major antigen lipoprotein (lpp^-). Small cell variants sensitive to pressure treatments contained the lipoprotein (lpp^+). The small cell variant-resting cell was not recognized by the immune response. C. Conditions for immunoblot. The conditions for the electrophoretic transfer of a family of antigenic lipoproteins from polyacrylamide gels to nitrocellulose paper was determined. D. Inactivation of C. burnetii. Enviro-Chem at a concentration of 5% effectively inactivated C. burnetii in 30 minutes. E. Significance. C. burnetii evades the immune response by sporulation events which result in neither the spore nor the small cell variant-resting cell being recognized as foreign by the infected host, thereby, establishing chronic infections. The detection of major antigens are possible with the establishment of new immunoblot procedures. Five percent Enviro-Chem will effectively inactivate materials contaminated with C. burnetii.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI 00417-06 OSD

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Systematics and Vector Relationships of Ticks (Ixodoidea)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James E. Keirans	Research Entomologist	OSD, NIAID
OTHERS:	Richard G. Robbins	Museum Specialist	OSD, NIAID

COOPERATING UNITS *(if any)* Dr. C. E. Yunker, Heartwater Project, Harare, Zimbabwe; Drs. D. Sonenshine and P. Homsher, Old Dominion University; Dr. J. B. Walker, Onderstepoort, R.S.A.; Dr. R. G. Endris, Plum Island Animal Disease Laboratory, USDA.

LAB/BRANCH
Office of the Director, Division of Intramural Research

SECTION
Entomology Department, Museum Support Center, Smithsonian, Washinggon, D.C.

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Project has been terminated.

ACB

ANIMAL CARE BRANCH

1989 ANNUAL REPORT

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ANIMAL CARE BRANCH

1989 Annual Report

Summary

Much of the Current research in the understanding and treatment of allergies and infectious diseases requires the use of laboratory animals. The care and treatment of these animals can have a profound affect on the results of experimental procedures. An animal's environmental conditions, its daily care, the presence or absence of disease-causing microorganisms, the amount of pain or distress that results from experimental manipulations all may affect the validity of research data. The NIAID intramural scientists today are very sensitive to these factors and their ethical responsibility for ensuring that animals used in research receive high quality animal care. All NIAID scientists conducting research that involves animals are required to attend the NIH course on "Using Animals in Intramural Research: Guidelines for Investigators." This course offers not only a broad perspective on current animal issues but also provides specific information on actual techniques and procedures.

It is the responsibility of the NIAID Animal Care Branch to provide the daily care to all animals maintained within the NIAID animal facilities, to oversee NIAID's intramural contracts involving animals, and to assist NIAID scientists with animal-related problems. The NIAID maintains four animal facilities at the NIH campus in Bethesda, Maryland, two animal facilities at the Frederick Cancer Research Facility in Frederick, Maryland, and four animal facilities at its' Rocky Mountain Laboratories at Hamilton, Montana. Approximately 54,000 square feet of space (22,000 sq. ft. at the NIH in Bethesda, MD; 2,500 sq. ft. at the FCRF in Frederick, MD; and 29,000 sq. ft. at the RML in Hamilton, MT), excluding pasture land, are devoted to the NIAID Animal Care Program.

The NIAID's Animal Care Branch provides guidance to the Institute's intramural scientists using animals in research projects. This guidance includes assistance in the purchase of animals, the selection and proper administration of anesthetics and analgesics, the diagnosis, treatment, and control of infectious agents, and the performance of technical procedures in laboratory animals. The Branch maintains production colonies of over sixty different strains of mice, hamsters, cotton rats, and rabbits for NIAID investigators within its' or contracts' animal facilities. Many of these animals are unavailable anywhere else in the world or are available only after long delays. The NIAID Division of Intramural Research is committed to the use of animals only when alternative methods are unavailable, and fully supports existing Federal rules and regulations pertaining to the care and use of animals in biomedical research. The Institute has hired three additional veterinarians to help manage its' animal care and use program and has spent, in FY89, over $500,000 in upgrading its' animal facilities and purchasing new animal equipment.

AMB

ADMINISTRATIVE MANAGEMENT BRANCH

ANNUAL REPORT FY 1989

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ADMINISTRATIVE MANAGEMENT BRANCH, DIR

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

October 1, 1988 to September 30, 1989

Introduction

FY '89 was a very challenging year for the Administrative Management Branch. We experienced an increased turnover in staff, extensive building/staff relocations, the necessity to implement many new procedures, and the implementation of numerous changes in procurement procedures and practices. Despite the numerous changes, AMB was able to make many advances in the areas of personnel, budget and financial management, procurement and contracts, renovations and space and automation.

Budget and Financial Management

In FY'89 DIR's budget allocation experienced an increase over FY'88 of 17% for a total of $90,805,000. In addition, in FY'89, 69% of DIR's total budget was allocated for research other than AIDS, while 31% of our budget was dedicated to AIDS research. AMB was responsible for monitoring and accounting for these funds separately and providing appropriate budget information for all DIR laboratories. The addition of AMBIS last year (in Building 10) has improved budget monitoring for the laboratory and administrative staff resulting in more comprehensive reports available for all staff.

Renovations and Space Relocations

The move from Building 5 to the newly renovated Building 4 was completed in December, 1989. The relocation involved the movement of approximately 265 individuals, 70,000 square feet of space which included scientific equipment and the LPD insectiary. Further, the move involved a great deal of coordination among scientific staff, the Divisions of Safety and Engineering Services, security, laboratory service and support companies, and transportation companies. Coordination of this move took tremendous administrative organization and effort. The administrative staff was able to successfully complete this move within targeted time frames, despite the numerous logistical problems that occurred. Additionally, after the move was completed the administrative staff spent an inordinate amount of time resolving building problems and staff concerns, including equipment hook up, renovation change orders, etc. Major coordinating efforts were also performed by the administrative staff in obtaining final clearance in March 1989 from the Divisions of Safety and Engineering for Building 5.

Construction is estimated to begin in July for BL 3 containment in 1100 square feet of laboratory space and 7500 square feet of animal space at the Twinbrook II facility. The expedited renovation of 3000 square feet of temporary laboratory space on the ground floor of Twinbrook was completed for the relocation of 16 staff members displaced from Building 5. Special arrangements were negotiated and organized with DES and the labs were renovated in six weeks!

During the past year the AMB worked closely with Program Resources, Inc. (PRI), to complete Phase II of the renovations to Building 469 in Frederick (FCRF). Currently, the AMB is working with the PRI contractors and NIAID scientists to develop a transgenic mouse facility and to correct heating and air conditioning problems with the newly installed HVAC system in Building 550.

Procurement/Contracts

In order to monitor $17 million in DIR funding allocated for contracts, AMB created a contract status and budget tracking report for all DIR supported contracts. These reports provide a monthly update on all commitments and obligations as well as projections of additional funding requirements for the fiscal year. The preparation of contract renewals must begin 18 to 24 months prior to the expiration of a contract and AMB has established a schedule for the renewal cycle of all DIR supported contracts to insure a smooth process from Board Concept Review through the final award. A new budget report has been developed to inform project officers of their commitments against contract funds. In an effort to better evaluate and report the performance of the FCRF contractors, a progress report has been developed for project officers to complete quarterly. Based on the information reported, NIAID will assess the merit of the contractors and recommend the percentage of the award fee to be granted. In continuing the process of providing guidance to the DIR scientific community, a Guide to Procurement was produced and distributed to the staff. This guide denotes the specific information which staff members must provide to insure all ordering is accomplished in a timely manner while adhering to all DHHS and NIH delegated procurement regulations.

Due to reports generated by the Inspector General and subsequently mandated by the NIH, we experienced a multitude of changes in procurement regulations resulting in the implementation of many additional procedures and regulations. Additionally, efforts were expanded in an attempt to further improve procurement within the DIR and to satisfy the needs of the laboratories. Several projects were initiated to implement changes with minimal impact on the scientific staff. In order to clarify several important procurement questions posed by the scientific staff and to provide information on changes in procurement guidelines, we enlisted the help of the Director, Delegated Procurement Program, NIH, to conduct procurement seminars for scientific staff, purchasing agents, and administrative officers. Staff survey responses from these seminars indicated a been very positive response to these sessions and the usefulness of the information provided.

To maintain a high standard in the area of procurement, the NIAID has established a Procurement Review Committee. With supervision from the NIAID Procurement Oversight Official (the Chief, AMB), this committee is charged with establishing and effecting internal reviews of delegated procurement functions. While not being labeled as an "audit", a team of one administrative officer and one senior purchasing agent review records, documentation, and procedures for each of the delegated procurement nodes within the NIAID. These internal reviews are intended to standardize NIAID procurement procedures and to afford a means of communicating areas requiring change or improvement as well as training. In addition, the DIR's Procurement Improvement Committee (PIC) also chaired by the Chief, AMB, was established with a mission to examine the present procurement system and to develop ideas for improvement within the guidelines of the NIH corrective action plan. Members of the committee include DIR technical, scientific and administrative staff. Even in its early stages of inception, this committee has become very instrumental in increasing laboratory staffs' appreciation of the way we "do business" and is fostering better relationships between the administrative and the scientific staff.

The NIAID Procurement Tips Newsletter, written and published by DIR administrative staff, has become a very useful tool for all NIAID staff. The newsletter provides information on current procedures, new regulations, etc. Complementing the newsletter, Purchasing Agents routinely conduct meetings with laboratory staff in their assigned area to provide updated information and to discuss new regulations and procedures. The "Procurement Catch Up Days" program was also implemented in the AMB this fiscal year. This program was developed to ensure that any Administrative Office experiencing procurement backlogs will receive the help of a Purchasing Agent(s) from another AMB Office. Finally, in an effort to broaden the administrative staff's understanding, to provide a clear picture of the relationship of their work to that of the scientific staff, and to foster better communications between all DIR staff, we also implemented mini-research seminars for the administrative staff. Routinely, a member of DIR's scientific staff presents a lecture to the staff (in laymen's terms) on their research.

Automation

The LAN staff consists of a computer specialist from ITEB, OAM, assigned to DIR; and one DIR full-time computer specialist. Additional support is provided by a contract programmer and two recently hired contract computer specialists.

The local area network (LAN) was expanded to include Bldg. 4 and the Rocky Mountain Laboratories in Hamilton, Montana. There are approximately 500 LAN users within DIR. With the installation of T1 lines (high speed telephone lines) and routers (connectivity hardware) at various LAN locations, the network has become a WAN (wide area network), thereby allowing users to access all network services and applications, not just those services and applications in their immediate domain, as well as enhancing network administration management.

Upgrades were made to the Employee Performance Plan System (EPMS), including options that allow plans to be sent to other network users and options to change the order of elements in the plans. The Acquisition Management and Budget Information System (AMBIS) underwent numerous enhancements in order to meet the needs of the lab and administrative staff. A few of the enhancements to AMBIS included downloading the BPA listing from DCRT weekly into the system, displaying a warning message when the laboratory is within 10% of their monthly trackable supplies and services allocation, creation of a list of Federal Supply Schedule items that have been ordered through the system, integrating the section vendor file with the NIH BPA list to provide lab personnel with information on whether the vendor is on the BPA list and if so whether they have items available on FSS, adding a utility to enable Purchasing Agents to divide orders, separating open market from FSS items. WordPerfect 5.0 was installed on the Network in June of this fiscal year.

A FAX gateway was installed, allowing users of the network to address network mail messages to be routed to facsimile machines. The NIH-MBUG sequence analysis package was modified for use on the network; and, at this time an experimental version is available for users connected via T1 lines. These programs allow the molecular biologist to examine protein and nucleic acid sequence data. Sequence similarity searches versus the PIR protein data base or the Gen Bank nucleic acid data base can be performed. Restriction site searches of nucleic acid sequences, translation of nucleic acid sequences to protein sequences, hydrophobicity and predicted secondary structure analysis of protein sequences and complex pattern matching studies are some of the functions performed by these programs.

Personnel

This fiscal year the AMB experienced a high turnover in staff. Two administrative officer positions and three Purchasing Agent positions became vacant. However, currently, these positions are filled and we are beginning to return to business as normal. In addition, one new Clinical Health Systems Specialist position was established to provide support services to the our clinical programs, especially the expansion of our AIDS clinical protocols. AMB's FTE ceiling increased from 25 in FY'88 to 28 in FY'89. We also have 1 additional Stay-in-School position increasing our total SIS positions to 4.

Two Administrative Technicians' positions (GS-303) have been reclassified to Administrative Assistant, GS 341 series, to allow them more opportunities for growth and career advancement. In November of this year, we added typing as a parenthetical title to our Purchasing Agents' position descriptions, allowing this series the same special pay rates enjoyed by clerical employees. Also in November of this year, AMB's Purchasing Agents received a group cash award for excellence in performing procurement functions and for the commendations received from the Logistics Management Institute (LMI) in their report to the NIH in April 1988. The Chief, AMB received the NIH Director's Award, one Administrative Officer received the NIH Merit Award and another Administrative Officer received the NIAID Director's Award. Overall, 87% of all AMB staff received some type of an award for exceptional performance or meritorious service this fiscal year.

In carrying out personnel policies and procedures, this year the administrative staff was faced with the task of devising and implementing internal procedures to handle a number of new personnel policies and procedures such as Medical Officers Retention Bonus (MORB), the Clinical Associate Program, Medical Staff Fellow $5,000 pay increases, new IMS travel program, Promotion Advisory Committee (PAC), Physicians' Licensure, Employee Survey, the AIDS Loan Repayment Program, and the NIAID Sabbatical Program for Minority Scientists.

In early 1987, at the request of the Director, NIAID, the Management Improvement Committee developed a questionnaire to be completed by every NIAID employee. The questionnaire asked each employee to identify strengths, point of specific problems hampering productivity and innovation, and recommend solutions. Analysis of the Employee Survey results indicated that follow-up was needed to refine and clarify issues, concerns, etc. In February 1989, Frumerman Associates, Inc. (a private firm contracted by the NIAID), provided the results of the NIAID Follow-Up Employee Survey. Although the general consensus of NIAID staff expressed pride and satisfaction in being a part of the NIAID, a number of areas of concern were also expressed. As a result, the AMB was charged with developing responses to the Survey and to provide a comprehensive corrective action plan for the DIR. The corrective action plan included several noteworthy initiatives that were implemented this fiscal year such as establishing bi-yearly meetings with the Director, DIR and each of the six groups identified in the Survey (e.g. technicians, supervisors, secretaries, etc.), reinstituting quarterly secretaries' meetings and monthly purchasing agent meetings with the Chief, AMB, revising procedures for GS 9-12 promotions, implementing the Procurement Improvement Committee (PIC), and providing formal training seminars on delegated procurement to laboratory staff. And finally, so that all staff could "shine their bright ideas" on the DIR and be heard, we purchased new free standing and creatively designed (light bulbs) suggestion boxes.

Through this challenging year, the Chief, AMB's committee involvement continued to increase. The Technology Transfer Advisory Committee was established by Dr. Fauci for FTTA and the Chief, AMB holds the Vice Chairperson position on this committee. Her work has continued to expand in the area of federal technology transfer, cooperative research agreements, and patent policy. She has also been responsible this fiscal year for writing the NIAID Patent Policy guidelines. In addition, she is rewriting the Patent Policy Training Book for upcoming November, 1989 training sessions. Besides the committees listed, she is also a member of the AIDS Management Team, Clinical Center Budget Review Committee, Friends of the Clinical Center, Intramural Administrative Officers, DES Subcommittee, Procurement Oversight Official for NIAID, Procurement Improvement Committee, promotion Advisory Committee, Technology Development Coordinator, Technology Managment Team, and the Committee to Write NIH's Manual Issuance on Sponsored Travel.

BRB

BIOLOGICAL RESOURCES BRANCH
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Annual Report
Biological Resources Branch
National Institute of Allergy and Infectious Diseases
October 1, 1988 To September 30, 1989

RESEARCH PROGRESS

The Biological Resources Branch investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and hemapoietic cell differentiation. In addition to this fundamental immunological research, the Branch provides protein sequencing, peptide synthesis and flow cytometry expertise and services for the NIAID.

STUDIES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules, as well as factors controlling the expression of class I molecules have been emphasized in this research.

Regulation of Expression of Class I Molecules. Classical class I molecules are found on nearly all cells in the body, whereas Qa/Tla region encoded molecules have a restricted tissue distribution. To investigate the cause of these different patterns of expression, the regulatory regions of Q10, a Qa region gene that is expressed only in liver, were compared with those of H-2L^d. Base differences in the class I regulatory element (CRE) of the Q10 gene were found to correlate with the inability of this gene to bind the rI nuclear factor. Conversion of either of these bases back to the H-2L^d form restored a region of dyad symmetry, and restored trascriptional activity as well as rI nuclear factor binding. These results indicated that the dichotomy between the widespread tissue expression of classical class I genes and the restricted tissue expression of Qa/Tla encoded class I genes is in part due to differences in the cis acting regulatory sequences that interact with transacting nuclear factors to direct transcription (Handy, Coligan).

Interaction Between T Cell Receptors and Class I Molecules. Cells secreting a genetically engineered soluble class I H-2K^b molecule were found to inhibit recognition of an H-2K^b reactive hybridoma by H-2K^{bm10} transfected cells. A peptide from the wild-type H-2K^b molecule spanning the region of the bm10 mutation specifically inhibited activation of the T cell hybridoma by H-2K^{bm10} cells, thus indicating that amino acid residues 163-174 of H-2K^b define a region important for T cell receptor binding. A calculated K_d of T cell receptor for soluble H-2K^b was 10^{-7} M, while the K_d for soluble peptide was 10^{-4} M (Maloy, Coligan).

Class I Alloantigenic Epitopes. A human monoclonal antibody, TrAH10, was shown to react with HLA-A3.1 but not HLA-A3.2. These two class I molecules differ at only two positions in their amino acid sequences - position 152 (Glu vs Val) and position 156 (Leu vs Gln), respectively. Using peptides and substitutions in class I molecules generated by oligonucleotide site-directed mutagenesis, the arginine residue at position 145 and the glutamic acid residue at position 152 of HLA-A3.1 were found to be crucial for the binding of the allospecific antibody. This data serves to further emphasize that the region between amino acid positions 140 and 175 in class I molecules is crucial for immune recognition (Maloy, Coligan).

MOLECULAR STUDIES ON T CELL RECEPTORS

Dual Specificity of TCR α/β Expressing Cells? A cytochrome c-specific, MHC-restricted T cell clone with two complete rearrangements of the same Vβ1 gene element was shown to express two different TCR- α/β heterodimers. Antipeptide sera specific for TCR Cβ1 and Cβ2 peptides each immunoprecipitated distinct disulfide-linked cell surface heterodimers. The clone was derived from immunized allogeneic chimeric mice, and displayed multiple Ia specificities, including the ability to recognize antigen in association with both I-E^k and I-E^s Ia molecules, as well as alloreactivity to the I-E^b molecule. It is being determined whether each receptor contributes independently to the overall specificity of the clone. (Ezquerra, Coligan).

Diversity of γ/δ T Cell Receptors. Anti-peptide sera specific for various TCR γ and δ chains were used to show that at least 4 molecular forms of the $\gamma\delta$TCR exist in the periphery. Unlike CD3$^+$, CD4$^-$, CD8$^-$ thymocytes, no peripheral cells of this phenotype were found to express α/β TCRs (Maloy, Coligan). Although CD4$^-$ and CD8$^-$ thymocyte populations were found to express both Cγ1 and Cγ4 TCR-$\gamma\delta$ heterodimers, the relative level of Cγ4 expressing T cells was significantly lower than that in peripheral organs. In addition, certain TCR $\gamma\delta$ heterodimers present in thymus development were not found in adult thymocyte or peripheral lymphoid populations. Together these finding indicate an ordered development of γ/δTCR expressing cells in the thymus and selective expression of distinct γ/δTCR subsets in peripheral lymphoid organs (Coligan).

Dendritic Epidermal T Cells Expressing $\gamma\delta$TCRs. Resident dendritic epidermal T Cells (DETC) were found to express predominately Cγ1 containing $\gamma\delta$TCRs (Coligan). However, cell lines derived form resident DETC were found to express both Cγ1 and Cγ4 type receptors that paired non-randomly with Vδ1 and Vδ6 chains, respectively. Sequence analysis of the δ chains revealed that signigicant diversity was generated by complex V-D-J rearrangement patterns. These complex rearrangement patterns distinguish DETC from early thymocytes and indicate that, if DETC are thymic derived, they originate from relatively mature thymic cells (McConnell).

Function of the $\gamma\delta$ T Cell Receptor. CD4$^-$, CD8$^-$ $\gamma\delta$TCR bearing T cell clones were isolated from the synovial fluid of a rheumatoid arthritis patient. These T cell clones were shown to respond specifically to mycobacterial antigens without MHC restriction. This is one of the first cases of a demonstrated specificity for $\gamma\delta$ T cells (Coligan, Koning).

SYNTHESIS OF PEPTIDE ANTIGENS

The major objective of this project is the chemical synthesis of peptides that correspond to portions of a native protein. These peptides are then used to mimic the reactivity of the native protein or are used to make antipeptide sera that will crossreact with the native protein. The peptides are synthesized for many different projects, each with its own specific objective; however, in general, the antipeptide sera are used as probes for a native protein or for a specific region of a protein, while the peptides that are not used to make antisera are used to identify and mimic epitopes recognized by antibody, T helper, or cytotoxic T lymphocytes (CTL). During the past year, peptides have been synthesized for 50 investigators in the NIAID (Maloy, Coligan). Synthetic peptides and antipeptide sera are being used in the manner described to investigate many different proteins that include (1) products of the mouse and human MHC, (2) human and murine T cell receptor proteins, (3) viral proteins from HIV-1, rotavirus, vaccinia virus and various strains of influenza virus, (4) parasite proteins from malaria, schistosoma and trypanosoma (5) various T cell proteins such as CD28 and integrins (6) newly described B cell proteins (7) prion proteins (8) zinc-finger proteins.

PROTEIN SEQUENCE ANALYSES

The facility has analyzed over 270 samples during the past nine months and projects that it will analyze about 340 samples this fiscal year from the following sources:

NIAID	Sample No.
LPD	23
LI	1
LVD	50
LIR	63
LCI	41
BRB	93
Sub total	271
NHLBI	3
NCI	6
Extramural	58
total	338

In addition to sequence analyses, in the case of ferrachelatase and a vaccinia protein, the facility has purified tryptic peptides for sequence analysis by reverse phase microbore HPLC separations. Several studies in which data provided by the facility have had a major impact are listed as follows: (1) a new serum protein tentatively thought to be a complement protein (P. Langlois, Carl Hammer, LCI); (2) VDP-galactosyl ceramide transferase produced from a tissue culture model (L. Vitovic, LIR); (3) vaccinia virus polymerase (B. Ahn, LVD); (4) tumor specific antigens (B. Seon, Roswell Park Memorial Institute); (5) ferrochelatase, an enzyme in the heme biosynthetic pathway that is deficient in protoporphyria (D.Brenner, UCSD); (6) Met-72 a protein from a melanoma cell variant (A. Kimura, University of Florida); and (7) TGFβ from xenopus XTC cells (M. Sporn, NCI and I. Dawid, NICHHD).

FLOW CYTOMETRY ANALYSIS

Over the past year, the flow cytometry section has accomplished the following: The FACS 440 has been upgraded, both the acquisition software and instrument modifications, to acquire a 5th parameter. This also includes addition of software to analyze list mode data. A krypton laser has also been added to the 440 to allow simultaneous UV and visible analysis (important for calcium studies on heterogeneous cell populations). Preliminary UV-calcium studies have begun on the FACS Analyzer. The MacIntosh II has been connected to the FCS computers via ethernet (using LAN calling) for analysis of data and transfer into MacIntosh compatible software. We have completed the initial phase of linking our IBM compatible PC to the FCS computers via ethernet (LAN cabling). The final phases will complete after installation of the recently purchased MicroVax 3000 scheduled for the end of FY'89 or early Fy'90. Two new large capacity disk drives and a large capacity archival tape storage device have been added to the MicroVax II computer, which serves as the primary acquisition and analysis computer of the FACStar Plus. This will allow for more efficient handling of data both for acquisition and analysis.

Biological Resources Branch
Annual Report
October 1, 1988 to September 30, 1989

ADMINISTRATIVE REPORT

This period marks the second year of the existence of the Biological Resources Branch. There are four Sections within the Branch; three of which are primarily involved with providing technological resources to the NIAID. Despite difficulties in keeping personnel trained in highly sophisticated techniques from being hired by other organizations, all of the service facilities have continued to be highly productive in supporting a diverse group of research projects within the DIR. The Molecular Structure Section has greatly benefited from the increased experience of Mark Garfield who has been senior technician in this facility for the past 18 months. In the Flow Cytometry Section, Vicki Ferretti-Aceto departed and the responsibility for running the BD model 440 Flow Cytometer was taken over by a newly hired, experienced technician, Susan Barbieri. The greatest personnel problems have been in the Synthetic Peptide Antigen Section. The Head of this section, Dr. W. Lee Maloy, departed for a position in private industry with much better benefits and salary and the senior technician in this Facility, Don Walker of PRI, took a job in private industry for similar reasons. The chaos generated by the departure of these key personnel has been reversed by the hiring of a peptide chemist, Dr. Jan Lukszo, and a new technician, Michael Knierman, to staff the peptide production facility within PRI at the FCRF. The transfer of Dr. Cliff Snyder, a protein chemist, into this unit has been key in stabilizing the production of peptides. The technician, Natalie Payne, supporting the research component of this section was also lured away by private industry and a new technician, Clifton West, has been hired and is being trained. Active recruitment to replace the Head of this Section is underway.

The Branch secretary, Iva DeArmon departed for more career potential and has been replaced by Ms. Valarie Sheppard. In the research area, Dr. Diane Handy left Dr. Coligan's laboratory after three and one half years to assume a tenure-track position in the Department of Medicine, Boston University Medical Center. Dr. Scott Wadsworth from the Department of Pathology and Laboratory Medicine of the University of Pennsylvania has recently joined Dr. Coligan's laboratory as an IRTA.

Biological Resources Branch
Annual Report
October 1, 1988 to September 30, 1989

HONORS AND AWARDS

Dr. Coligan was invited to present a laboratory data seminar at the Dana Farber Cancer Institute. He was also invited to make presentations at the FASEB meeting in New Orleans, the International Congress of Immunology in Berlin and the UCLA Symposium on Immunogenicity. Dr. Coligan continues to serve on the editorial boards of Molecular Immunology and Immunologic Research. Dr. Coligan received a DHHS award for Recognition and Special Achievement and Dr. Kevin Holmes was presented with the NIAID Merit Award. Dr. Holmes also made a presentation at the International Congress of Immunology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00169-12 BRB

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan Branch Chief BRB, NIAID
Others: Diane Handy Staff Fellow BRB, NIAID

COOPERATING UNITS *(if any)*

W. Biddison, NINCDS, NIH; D. Margulies, NIAID, NIH; K. Ozato, NICHHD, NIH

LAB/BRANCH

Biological Resources Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Major histocompatibility complex encoded class I molecules are integrally involved in the presentation of viral antigens to cytotoxic T lymphocytes (CTL). The goal of these studies is to gain an understanding of the regulation of expression, and functional and antigenic-properties of these molecules. Recent studies have focussed on gene regulation. It was shown by gel retardation analysis that the nuclear factor (rI) that binds to the inverted repeat (TGGGGATTCCCCA) of the class I regulatory element (CRE) present in classical class I genes such as H-2L^d does not bind to the equivalent region of the Q10 liver-specific class I gene. Both the Q10 and H-2L^d CREs bound the rII nuclear factor. The Q10 CRE differs from that of classical class I genes in two bases that disrupt the region of region of dyad symmetry. Using site specific in vitro mutagenesis to individually change these two nucleotides, we were able to show that both were involved in the binding of the rI nuclear factor. These results indicate that binding of the rI nuclear factor to the CRE is important for the broad tissue type expression of classical class I molecules and that alterations in the CRE that inhibit rI binding relate to the tissue specific expression of certain class I molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00352-07 BRB

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John E. Coligan	Branch Chief	BRB, NIAID
Others:			
	Angel Ezquerra	Visiting Fellow	BRB, NIAID
	Kevin Moulder	Visiting Fellow	BRB, NIAID
	Gary Kikuchi	NRC	BRB, NIAID
	Gregory Einhorn	IRTA	BRB, NIAID
	Tom McConnell	Guest Worker/NRC	BRB, NIAID

COOPERATING UNITS *(if any)*

E. Shevach, LI, NIAID; Goerg Stingl, Vienna University, Austria; Jeff Bluestone, University of Chicago

LAB/BRANCH

Biological Resources Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	5.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Biochemical analysis of CD3^{+}, CD4^{-}, CD8^{-}T cells present in peripheral lymphoid organs were shown to express a variety of γδTCR proteins of either the Cγ1/2-type or Cγ4 -type. Unlike CD3^{+}, CD4^{-}, CD8^{-} thymocytes, these peripheral cells were shown to express high levels of Cγ4 γ/δ TCRs, but little, if any TCRαβ. Biochemical analysis of adult and fetal thymocytes revealed the presence of T cells expressing Cγ1- and Cγ4-type γδ TCR. The relative level of Cγ4-expressing T cells was significantly lower than that observed in peripheral lymphoid organs. In addition, biochemical studies revealed the presence of γδ TCRs expressed during fetal ontogeny that were not detected in adult thymocytes or peripheral lymphoid populations suggesting the selective expression of distinct TCR-γδ subsets in peripheral lymphoid organs.

Most resident dendritic epidermal T cells (DETC) were shown to express a TCR γδ of the Cγ1 type. This is in contrast to cell lines derived from this population of which a significant number express Cγ4 type receptors. However, the demonstration of TCR heterodimers on resident Thy-1^{+} DETC strongly implies that these cells are functional T cells. The diversity of δ-gene expression by DETC lines was analyzed by Northern and Southern hybridization analysis as well as cDNA sequence analysis. Only Vδ1 and Vδ6 usage was detected and these δ chain types were found not to pair randomly with γ chains. Vδ1 chains paired exclusively with vδ3/Cγ1 chains and vδ6 with Cγ4 and some Cγ1 chains. Complex V-D-J gene rearrangement patterns were shown to distinguish DETC cells from early thymocytes suggesting that, if they are thymus-derived, they originate from relatively mature thymic cells. DETC cells were also shown to express CD-28 and integrin-like molecules that appear to be involved in their activation and function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00543-02 BRB

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Recognition of Peptidic Antigens by Virus-Specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John E. Coligan	Senior Investigator,	BRB, NIAID
Others:	Robert W. Anderson	IRTA Fellow	BRB, NIAID
	W. Lee Maloy	Senior Investigator	BRB, NIAID

COOPERATING UNITS *(if any)*

Bill Biddison, NI, NINCDS; D. Margulies, LI, NIAID

LAB/BRANCH

Biological Resources Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
3.2	1.5	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It was demonstrated that stimulation of an H-$2K^b$-reactive T cell hybridoma can be inhibited by either genetically engineered soluble H-$2K^b$/ $Q10^b$ or by a synthetic peptide identical to H-$2K^b$ between amino acids 163 and 174. Inhibition by the soluble protein was seen under conditions of suboptimal stimulation of the T cell hybridoma by a weakly cross-reactive ligand, H-$2K^{bm10}$. Both coculture of the T cell hybridoma with cells secreting the soluble H-$2K^b$/$Q10^b$ or with immunoadsorbent purified H-$2K^b$/$Q10^b$ protein inhibited IL-2 release when the T hybridoma cells were suboptimally stimulated. Half-maximal inhibition for the purified protein was seen at a concentration of approximately 10^{-7} M. In contrast, half-maximal inhibition by H-$2K^b$ peptide 163-174 was observed at a concentration of approximately 10^{-4} M.

Analysis of the specificity of the HLA-A3.1 specific human antibody TrAH10 demonstrated that it binds an epitope that is located between amino acid residues 144 and 160 on the HLA-A3 α2 helix. Several lines of evidence indicated that the arginine residue at position 145 and the glutamic acid residue at position 152 were critical for the formation of the epitope.

These studies serve to emphasize the importance of the region between positions 140 and 175 of the class I molecule for immune recognition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00523-02 BRB

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Flow Cytometry Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Kevin Lee Holmes Senior Staff Fellow BRB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biological Resources Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS 4.5	PROFESSIONAL 5	OTHER 4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The Flow Cytometry Section has increased its ability to acquire multiparameter data by upgrading the FACS 440 to acquire and analyze a 5th parameter, including the addition of a laser for UV-calcium uptake studies. The FACS Analyzer is currently being used for UV- calcium studies, one of the original purposes for acquiring the instrument. The initial stages of linking the DIR PC's via the LAN, both MacIntoshes and IBM compatible, to FCS computers has been completed. Further progress in this area is forthcoming in FY'90. The overall usage of FCS instrumentation has increased in FY'89, totaling 3478 hrs or 10 hrs/day of instrument time by DIR investigators. This represents an effective use of instrument time throughout FY '89.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00172-11 BRB

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Synthesis of Peptide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: W. Lee Maloy/John E. Coligan Senior Investigator BRB, NIAID

COOPERATING UNITS *(if any)*

John E. coligan, BRB, NIAID; Louis Miller, LPD, NIAID; Ronald Schwartz, LI, NIAID; David Margulies, LI, NIAID, Mario Gorziglia, LID, NIAID

LAB/BRANCH

Biological Resources Branch

SECTION

Synthetic Peptide Antigen Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

During the past year, over 350 peptides have been synthesized for members of the NIAID DIR for use in many different projects. These studies have been reported in over twenty papers in major scientific journals. One of the most successful collaborations has been with the Malaria Section of LPD. These studies have used synthetic peptides to characterize helper and cytotoxic T cell epitopes in the circumsporzoite protein of Plasmodium falciparum malaria. It has been determined that the epitopes recognized in both mice and human are in a polymorphic area of the protein and in most cases the different epitopes are not cross-reactive. It was also observed that recombinant IL-2 could overcome Ir gene control, producing strong immune responses to circumsporozoite peptides in low responder mice.

In a collaborative study with the LID a synthetic peptide was used to define a neutralization epitope in the VP3 protein of the human rotavirus. This information will be important in the development of vaccines against human rotavirus infection.

Significant studies were also carried out using synthetic peptides on 1) the structure, biosynthesis and expression of the T cell receptor complex 2) the presentation of peptides antigens to influenza specific cytotoxic T lymphocytes; and 3) the mechanism of recognition by allo specific cytotoxic T lymphocytes. These studies are described in detail in other project reports contained in this annual report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00522-02 BRB

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Sequence Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan Branch Chief BRB, NIAID

COOPERATING UNITS *(if any)*

P. Gershon, LVD, NIAID; B. Seon, Roswell Park Memorial Inst.; A. Kimura, Univ. Florida; D. Brenner, UCSD; M. Sporn, NCI

LAB/BRANCH

Biological Resources Branch

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year, the amino acid sequences of over 330 proteins and peptides have been determined by automated protein sequence analysis. In the cases of ferrochelatase and certain vaccinia virus proteins, the facility has purified tryptic peptides for sequence analysis by reverse phase microbore HPLC separation. Some of the studies impacted by this work are listed: 1) Characterization of a new complement protein (Laboratory of Clinical Investigation); VDP-galactosyl ceramide transferase produced in a model system (Laboratory of Immunoregulation); Vaccinia virus proteins (Laboratory of Viral Diseases); Tumor specific antigens (Roswell Park Memorial Institute and University of Florida); TGFβ from xenopus (NCI and NICHHD); and ferrochelatase (UCSD).

LCMI

Laboratory of Cellular and
Molecular Immunology
1989 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1988 through September 30, 1989

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up in vitro analogs in tissue culture, and determine the molecular basis for the phenomena. The laboratory employs techniques form the disciplines of cellular immunology, molecular biology and protein biochemistry.

During its third year the laboratory has pursued three major areas of research: 1) antigen recognition by γδ T cells, 2) the biochemical basis of T cell activation and inactivation, and 3) the intrathymic events in T cell differentiation.

Antigen Recognition By γδ T Cells

Two distinct subpopulations of thymus-derived (T) lymphocytes exist: one bearing the αβ T cell antigen receptor and the other bearing the γδ T cell antigen receptor. These two subpopulations represent distinct lineages of cells that arise at different times in thymic development. αβ T cells recognize peptides derived from foreign proteins bound to MHC class II molecules on the surface of an antigen-presenting cell. The nature of the ligand recognized by the γδ T cell receptor is unknown. The current work represents an effort to find a ligand for the γδ T cell and to characterize the nature of the antigen presentation.

Intrigued by a report of increased number of $CD4^-CD8^-$ T cells (the phenotype of γδ T cells) in a tuberculosis pleural effusion, we investigated the possible role of γδ T cells in the primary immune response to Mycobacterium tuberculosis. Immunization with Freund's complete adjuvant (containing a mycobacterial extract) resulted in a 24-fold increase in the $CD4^-CD8^-$ T cell subpopulation. This expansion did not occur with Freund's incomplete adjuvant, suggesting that the mycobacterial extract was a key component for eliciting the response. Three biochemically distinct γδ T cell receptors were detected in the purified $CD4^-CD8^-$ T cell subpopulation isolated in vitro. Analyses of cell cycle, of interleukin-2 receptor expression, and of interleukin-2 responsiveness showed that a large proportion of the γδ T cells were activated in vivo. The purified T cell receptor γδ cells responded to solubilized Mycobacterium tuberculosis antigens in vitro, but, in contrast to Mycobacterium tuberculosis-specific αβ T cells, the response of γδ T cells to Mycobacterium tuberculosis did not require recognition of major histocompatibility complex class II molecules. These results provide an example of antigen-specific activation of γδ T cells in vivo, and indicate that γδ T cells may have a distinct role in generating a primary immune response to certain microorganisms.

(E. Janis, Z. Kovac, A. Bendelac, P. Bacon, and R.H. Schwartz; LCMI/NIAID, D.M. Pardoll, Dept. Med., Johns Hopkins; S.H.E. Kaufmann, Institute for Microbiology, Univ. of Ulm).

The Biochemical Basis of T Cell Activation and Inactivation

T lymphocytes of the αβ lineage are activated to release lymphokines and divide after recognizing a peptide derived from a foreign antigen bound to a major histocompatibility complex (MHC) - encoded molecule on the surface of a second cell type, the antigen-presenting cell (APC). Recent studies suggest that the APC not only displays the antigen for the T cell to recognize, but it also provides a second signal required for activation. Without this costimulatory signal our studies suggest that the T cell is driven into a nonresponsive state in which it can no longer be activated to make the autocrine growth hormone interleukin-2. Our major objectives during the past year were to rigorously test this two signal model for T cell activation using several new stimuli and determine whether the costimulatory signal was absolutely required for activation of IL-2 production.

Others have shown that both anti-CD3 monoclonal antibodies, which react with the conserved ∈ chain of the T cell antigen receptor, as well as a combination of a phorbol ester (PMA) and a calcium ionophore (ionomycin), which are thought to mimic receptor-induced second messengers that lead to the activation of protein kinase c and increases in intracellular free calcium ions, respectively, stimulate resting T lymphocytes to divide. These signals were thought to solely mimic T cell antigen-receptor occupancy and thus should have induced the nonresponsive state according to our model. In the past year we have examined both of these stimuli and found that their activation properties are cell density dependent. At low cell numbers the T cells do not divide unless provided with a source of costimulatory activity. This suggests that at high density a cryptic source of costimulatory activity, e.g., small numbers of contaminating antigen-presenting cells, accounts for the proliferative response. Thus, we can now conclude that the costimulatory signal is absolutely essential for the generation of a T cell proliferative response.

(D.L. Mueller, B. Beverly, S. Stringfellow, E. Fuchs, and R.H. Schwartz, LCMI/NIAID)

T Cell Differentiation

This project is aimed at defining and characterizing the series of developmental events involved in the generation of mature, immunocompetent T cells, and the selective events which determine the antigen-recognition repertoire of these cells. These studies primarily utilize the thymus, the major site of T cell differentiation in the animal. In the past we have identified a number of thymocyte cell types and made possible the isolation of these cells at very high purity on a preparative scale. We have used these isolated cell types in an effort to define the state of maturation, precursor-product and lineage relationships, expression of T cell receptors, and the mechanisms and inductive signals responsible for growth, differentiation, and selection in the thymus.

During the past year we have analyzed radiation-induced bone marrow chimeras for the development of tolerance to self antigens. In particular we have asked whether the thymic epithelial cells can contribute to this process. Using the Mls antigen we have demonstrated that the thymic epithelial cells express the antigen, that they shed it, allowing bone marrow-derived dendritic cells to pick it up and induce clonal deletion of T cells reactive against the antigen, but that the thymic epithelial cells themselves do not induce clonal deletion. Instead they impart a type of clonal anergy in which the T cells still exist but can no longer be activated to divide by stimulation with anti-receptor antibodies or the Mls antigen in vitro.

In a continuation of studies inhibiting T cell development with monoclonal antibodies against the CD4 and CD8 accessory molecules, we have now examined antibodies whose isotypes do not lead to cytotoxic or phagocytic elimination of T cells in the peripheral lymphoid organs. These antibodies still block the transition from double positive ($CD4^+CD8^+$) to single positive ($CD4^+8^-$ and $CD4^-8^+$) cells in the thymus, suggesting that the accessory molecules are required for the positive selection event in thymocyte development.

Finally, the single positive thymocytes found predominantly in the medulla of the thymus have recently been subsetted into two subpopulations based on the cell surface markers Qa2 and HSA. Both subsets (HSA^+ $Qa2^-$ and HSA^-Qa2^+) express high levels of the T cell antigen receptor but our analysis shows that they are different functionally. HSA^-Qa2^+ cells resemble peripheral T cells in that they proliferate in response to anti CD3 or anti-receptor antibodies (in the presence of added accessory cells). In contrast the HSA^+ $Qa2^-$ subset does not proliferate to the same stimuli because of a failure to produce IL-2. This subset appears earlier in development than the HSA^- $Qa2^+$ subset, suggesting that the HSA^+ $Qa2^-$ cell may be a functionally immature precursor of the HSA^- $Qa2^+$ cell.

(F. Ramsdell, E. Robey, B.J. Fowlkes LCMI/NIAID)

Administrative, Organizational, and Other Changes

During the past year Drs. Ellen Robey, Delanie Cassell and Calman Prussin joined the laboratory. Dr. Paul Bacon left the laboratory after completing a one year Sabbatical.

Honors, Awards, and Scientific Recognition

Dr. Schwartz is a member of the editorial board of Science, International Immunology, Immunology Today, The Journal of Molecular and Cellular Immunology, and the International Journal of Cell Cloning. During the past year he was a distinguished visiting scientist at the University of Texas Southwestern Medical School in Dallas, TX, a symposium chairman at the UCLA symposium on Immunogenicity in Steamboat Springs, CO, a symposium speaker at the 73rd Annual FASEB meeting in New Orleans, LA, a speaker in the Tolerance Workshop in Madrid, Spain, a distinguished lecturer at Albert Einstein College of Medicine in the Bronx, NY, a speaker at the Cold Spring Harbor symposium on Immunological Recognition in Cold Spring Harbor, NY, and a symposium speaker in the 7th International Congress of Immunology in Berlin. In addition, he was invited to give seminars on the work being conducted in his laboratory by the Immunology group at the University of California in San Francisco, Ca, the Department of Pathology at Harvard Medical School, Boston, MA, and the Howard Hughes Immunology group at Yale University Medical School, New Haven, CT.

Dr. B.J. Fowlkes was a workshop chairperson at the UCLA symposium on Immunogenicity at Steamboat Springs, CO, a symposium chairperson at the 73rd Annual FASEB meeting in New Orleans, LA, a session chairperson at the FASEB summer research conference on lymphocytes and antibodies at Saxton River, VT, and a symposium speaker at the 7th International Congress of Immunology in Berlin. During the year she was invited to give seminars on her work by the Institute for Cancer Research at Columbia University in New York City, NY, the Immunology Affinity Group at Scripps Clinic and Research Foundation in La Jolla, CA, the Department of Biochemistry at George Washington University in Washington, D.C., and the Department of Medicine and Pathology at Washington University in St. Louis, MO.

Dr. Daniel Mueller was a symposium speaker in the UCLA symposium on Immunogenicity held at Steamboat Springs, CO.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00529-02 LCMI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ronald H. Schwartz	Chief	LCMI, NIAID
Eric Janis	HHS	LCMI, NIAID
Albert Bendelac	VF	LCMI, NIAID
Paul Bacon	VS	LCMI, NIAID
Zdenko Kovac	VA	LCMI, NIAID

COOPERATING UNITS *(if any)*

D.M. Pardoll-Department of Medicine, Johns Hopkins University School of Medicine
S.H.E. Kaufmann-Institut Fur Mikrobiologie der Universitat Ulm

LAB/BRANCH
Laboratory of Cellular and Molecular Immunology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the immunologic role of T cells bearing the conventional αβ T cell receptor has been well characterized, little is known about the function of the population of T cells bearing the γδ T cell receptor. During the past year we have investigated the role of γδ T cells in the immune response to Mycobacterium tuberculosis. The number of T cell receptor γδ cells in the draining lymph nodes of mice immunized with Mycobacterium tuberculosis was greatly increased in comparison with the number of T cell receptor αβ cells. Three biochemically distinct γδ T cell receptors were detected. Analyses of cell cycle, of interleukin-2 receptor expression, and of interleukin-2 responsiveness showed that a large proportion of the γδ T cells were activated in vivo. T cell receptor γδ cells responded to solubilized Mycobacterium tuberculosis antigens in vitro but, in contrast to Mycobacterium tuberculosis -specific αβ T cells, the response of γδ T cells to Mycobacterium tuberculosis did not require major histocompatibility complex class II recognition. These results provide an example of antigen-specific activation of γδ T cells in vivo and indicate that γδ T cells may have a distinct role in generating a primary immune response to certain microorganisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00485-03 LCMI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
T Cell Activation

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: Ronald H. Schwartz	Chief	LCMI, NIAID
Daniel Mueller	MSF	LCMI, NIAID
Bart Beverly	IRTA	LCMI, NIAID
Steven Stringfellow	MSF	LCMI, NIAID
Ephraim Fuchs	MSF	LCMI, NIAID

COOPERATING UNITS (*if any*)

None

LAB/BRANCH
Laboratory of Cellular and Molecular Immunology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	4.5	2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Antigen-presenting cells serve two functions; they display peptides derived from the antigen on the cell surface for T cell recognition, and they provide a costimulatory signal required for T cell activation. Our previous studies have suggested that T cell recognition in the absence of the costimulatory signal drives the cell into a state of hyporesponsiveness in which it can no longer be activated to make the interleukin-2 required for division. Others have shown, however, that anti-CD3 monoclonal antibodies or phorbol esters and ionomycin stimulate resting T lymphocytes to divide. These signals were thought to solely mimic T cell antigen-receptor occupancy and thus should have induced the nonresponsive state according to our model. In the past year we have examined both of these stimuli and found that their activation properties are cell density dependent. At low cell numbers the T cells do not divide unless provided with a source of costimulatory activity. This suggests that at high density a cryptic source of costimulatory activity e.g. small numbers of contaminating antigen-presenting cells, accounts for the proliferative response. Thus, we can now conclude that the costimulatory signal is absolutely essential for the generation of a T cell proliferative response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00486-03 LCMI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
T Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B.J. Fowlkes	Senior Investigator	LCMI, NIAID
Fred Ramsdell	IRTA	LCMI, NIAID
Ellen Robey	Guest Researcher	LCMI, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Molecular Immunology

SECTION
Section on Thymocyte Differentiation

INSTITUTE AND LOCATION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analyses using radiation bone marrow chimeras for the development of tolerance to self antigens reveal that clonal deletion by the Mls antigen can occur by cross-presentation, that the Mls antigen is expressed by thymic epithelium, and that clonal deletion due to Mls or class II antigens is mediated only by bone marrow-derived elements and not by thymic epithelium. These experiments also demonstrate that, in the absence of clonal deletion, potentially self-reactive clones are tolerized by another mechanism, which results in clonal anergy. We are defining the stages of T cell development which serve as targets for tolerance induction by clonal deletion or clonal anergy. Single positive thymocytes ($CD4^+8^-$ and $CD4^-8^+$) express high levels of CD3 but are heterogeneous with respect to phenotype and functional competence. An investigation of these subsets for tolergenic capacity is in progress.

A project has been completed using in vivo antibody treatments to demonstrate that the CD4 and CD8 accessory molecules are required for positive selection of developing thymocytes into the appropriate T cell lineage.

LCI

LABORATORY OF CLINICAL INVESTIGATION
1989 ANNUAL REPORT
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Laboratory of Clinical Investigation

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Summary of Report
LABORATORY OF CLINICAL INVESTIGATION
October 1, 1988 to September 30, 1989
Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

INTRODUCTION

This has been an extremely exciting and productive year in the history of the Laboratory of Clinical Investigation. Many of the projects initiated in past years have begun to see their fruition. This includes many long-term projects with important clinical application. Members of the Senior Staff continue to be internationally recognized and the graduates of our program continue to do exceedingly well.

INFECTIOUS DISEASES GROUP:

MEDICAL VIROLOGY SECTION

A highly fundamental and clinically very important finding of Dr. Stephen Straus's group has been their observation on the basis of viral latency. Dr. Ken Croen in that laboratory has been able to show that Herpes Simplex virus (HSV) can be found in the ganglion cells of patients with latent HSV infection. Interestingly, Varicella Zoster virus (VZV) is not found in ganglion cells as had been expected but in accessory cells in the nerve ganglia. This provides an important insight into the difference in infection with these two agents and may explain the major difference in the clinical consequences of reactivation. In the case of HSV1 it was shown that there are viral transcripts of usually untranscribed DNA. These transcripts for what is in effect "antisense protein" may be of fundamental importance in determining the cause of latency. These studies continue.

Work in the laboratory has lead to major advances in mapping the genes and proteins present during active, latent and productive VZV infections. With VZV major gene proteins are being identified and mapped. The emphasis at the present time is in determining which transcripts are expressed during latency as a way of further defining this pathophysiologic process. Dr. Ostrove and collaborators in the group have been studying the role of Herpes viruses as cofactors in the progression and exacerbation of human immunodeficiency virus. It has been shown that HSV1 and 2 stimulate the synthesis of HIV and the specific HSV genes responsible for this effect have been determined. Detailed studies of this effect indicate that cellular proteins also contribute to the process of HIV activation.

Clinical studies in the laboratory have lead to further understanding of the treatment of genital herpes and its exacerbations. The group in participating in a multicenter trial of acyclovir for prophylaxis of herpes genitalis in a high risk population and will attempt vaccine studies with recombinant HSV protein antigens to determine whether or not this infection can be controlled. Clinical studies also examine the natural history of herpes simplex infections. Receiving a great deal of attention is the studies of the group in Chronic Epstein-Barr virus infection and its possible association with the Chronic Fatigue Syndrome. The group had earlier shown that acyclovir did not provide effective treatment for Chronic Fatigue Syndrome, even in patients with abnormal immune responses to EB virus demonstrated by serologic studies. More recently, the group has used more sophisticated in situ hybridization techniques to study EBV gene expression in these patients and the actual syndrome has been further defined. The group is in the midst of studies of the pituitary adrenal axis in these patients and have found statistically significant decreases in corticotropin releasing substances circulating in blood of these patients. This will be further explored with specific studies in which this system is directly stimulated.

BACTERIAL DISEASES SECTION

Our group that is interested in phagocyte function has also had a highly productive year. Drs. Malech, Gallin, and colleagues have made an important series of findings that relate to a number of aspects of neutrophil response. Thus, for example, working with Drs. Lomax and Leto they have succeeded in cloning two of the cytosolic factors responsible for the oxidative burst of neutrophils. They have studied these factors in patients with Chronic Granulomatous Disease, an inherited infectious disease in which patient's neutrophils do not respond normally with an oxidative burst following phagocytosis and thus do not kill intracellular organisms normally. They have shown that these patients can be divided into groups depending on the identifiable inherited defects in the various proteins that are required for the oxidative burst. Thus the group has made major advances in understanding the oxidative function of neutrophils and in relating these oxidative proteins to human disease. They have cloned and sequenced the proteins responsible for these functions and have provided chromosomal localization. They have also advanced their understanding of G protein function and have begun cloning of receptors for other factors that trigger neutrophil function such as chemotactic receptors and receptors for the complement fragment C5a.

Clinically, members of the group along with Dr. David Melnick have shown that gamma interferon increases the killing activity of cells from patients with Chronic Granulomatous Disease, particularly those associated with an autosomal form of inheritance. The latter is in turn associated with failure to form some of the cellular factors discussed above. A gamma interferon trial for treatment of this patient population is under way. It has also been shown that low dose glucocorticoids may be very effective in treating some of the features of this disease that are produced by obstructive granuloma.

Yet another group of patients with phagocytic defects, those associated with hyperimmunoglobulin E in the plasma and recurrent infections (Job's Syndrome) have also been studied. B cells from these patients were shown to synthesize increased amounts of IgE when cultured and in some patients' cells decreased their IgE production when gamma interferon was added to culture. Several patients have shown a decrease in their IgE levels following gamma interferon therapy although this is not a uniform finding. Gamma interferon was also found to induce monocyte polykaryon formation in an in vitro model of granuloma formation.

CLINICAL MYCOLOGY SECTION

The Clinical Mycology section under Dr. Bennett has continued its studies of host defense against yeast infection. Studies have been conducted to determine the sites of binding of Aspergillus fumigatus conidia to human pulmonary alveolar macrophages. It was found that a trypsin sensitive site on the macrophage recognizes a beta 1-3 glucan configuration on the conidia leading to binding. Macrophage CR3 was not involved. In studies in the laboratory it was also shown that deoxycholate amphotericin B when injected into patients causes fever. This pyrogenicity correlates with TNF alpha and IL-1 in vitro. It was found that normal neutrophils can cooperate with CGD neutrophils in killing Aspergillus hyphae in vitro. The normal cells provide oxidative products to the abnormal cells allowing them to continue the killing process. This provides a theoretical justification for the use of white cell transfusions is patients with Chronic Granulomatous Disease.

Dr. Kwon-Chung, in this group has continued her studies of Candida albicans and related species as well as Cryptococcus neoformans. Her group has cloned one of the virulence factors from the Candida and have determined its mechanism of action. They have shown that the types of Candida that cause disease in AIDS patients do not differ from those found in normal volunteers suggesting that these organisms establish themselves as pathogens because of defects in the host. The group is undergoing a careful molecular study of the differences between virulent and avirulent Candida species in an attempt to determine the mechanism of virulence with this important pathogen.

IMMUNOLOGY GROUP:

CLINICAL IMMUNOLOGY SECTION

The Clinical Immunology Section has had one of its most productive years as well. Possibly the most exciting finding of the group is the isolation of a new plasma protein. In studies of the purification of C2 a co-isolating protein that binds to activated C4 was found. This protein has now been cloned and partially sequenced. It represents a new plasma protein which is present in large quantities; all individuals studied thus far have about 300 micrograms per ml. The protein is a poor immunogen which explains why it was never previously discovered. This protein interacts with the complement cascade but also interacts with the kinin generating system and is capable of generating fragments that cause many of the same effects as bradykinin including capillary leakage and gut contraction. The group is interested in determining whether this protein may be responsible for the angioedema in hereditary angioedema and its other biological effects. Much of the group has devoted its attention to determining the structure and function of this protein which is very difficult to work with.

A second major discovery of the group this year relates to their findings with regard to the mechanism of action of intravenous immunoglobulin, an agent now used to treat Idiopathic Thrombocytopenic Purpura, Autoimmune Hemolytic Anemia and Kawasaki's disease. Drs. Fries and Frank working with a Visiting Fellow, Dr. Basta have explored the possibility that intravenous immunoglobulin produces its biological effect by intercepting activated complement components before they can attack a target and thereby preventing complement attack. Thus, intravenous immunoglobulin would be the first agent to be shown to function clinically by interrupting the complement cascade or at least the effectiveness of complement attack. In a red cell clearance model and in an animal model termed Forssman shock this was found to be the case with intravenous immunoglobulin having a profound effect on complement function. This finding raises a whole series of diseases that may be treated with this very safe therapeutic modality.

In a third study, Ms. Gaither and colleagues have found that patients with acute infections have major changes in their phagocyte function and have documented those changes. One of the more interesting findings was markedly increased phagocytosis of IgG coated particles without a major change in the number of IgG receptors on the phagocytic cells and phagocytosis via CR3 without a change in the number of CR3 on the cells. These studies also document important kinetic changes in the process of phagocytosis during infection. These studies go far to explain how cytokines released during the inflammatory and immunologic event arm cells to fight infection and document this effect in clinical situations.

One of the functions of the oxidative burst of cells is to form HOCl. HOCl has been shown by members of the group to down regulate Fc receptor function without causing either internalization or shedding of the Fc receptor. Recent studies have shown that this alters a binding site on the Fc receptor molecule decreasing its activity, although it remains present on the cell surface in an inactive form. This raises important questions about pathophysiologic mechanism of phagocyte function.

The group is interested in the mechanism by which antibody directs complement attack. They have turned their attention to complement attack on viruses in the last several years because viruses are simple particles to study. Their studies indicate that parainfluenza virus may activate complement in the absence of virucidal antibody but that the binding of complement does not cause viral neutralization. Virucidal antibody can allow complement to neutralize the virus. The thrust of the experiments now is to determine what aspect of complement attack is influenced by the presence of the antibody.

In clinical studies, the group has been interested in the treatment of Hereditary Angioedema and in defining a disease caused by the production of an autoantibody to the C1 inhibitor. Some patients with Hereditary Angioedema, a life threatening swelling disorder, respond to no therapy. A clinical trial of the usefulness of gamma interferon in these patients has been initiated. Although all patients demonstrate an increase in the level of C1 inhibitor, thus far there have been no cures and major toxicity of the interferon has been noted. The group has succeeded in developing a new simple method for the purification of C1 inhibitor that may well be commercially applicable.

MUCOSAL IMMUNITY SECTION

The Mucosal Immunity Section under Dr. Warren Strober has continued its important studies of the regulation of IgA immunoglobulin synthesis. The work of the last several years has shown the importance of various cytokines in regulating immunoglobulin class switching and in controlling immunoglobulin synthesis. In studies of Peyer's Patch B cells derived from human bowel wall and depleted of T cells it has been shown that IL-1 plus IL-6 supports enhanced IgM and IgG-3 synthesis but does not effect IgA synthesis. On the other hand, IL-5 plus IL-6 increases IgA synthesis enormously without effecting IgM or IgG production greatly. To do this work, recombinant proteins have been required and many of these recombinant proteins are produced in Dr. Strober's section. Dr. Strober's group also conducts important studies on the effect of cholera toxin and other factors that affect cellular metabolic systems on the production of IgA and on IgA cell switching. The latter point refers to the ability of bowel B cells to switch their immunoglobulin production from IgG to IgA production. The ultimate goal of these studies is to understand the factors that control the production of these immunoglobulins as a first step in understanding the mechanism of protection across mucosal barriers. The group has also isolated a new class of T cells present in the germinal centers of Peyer's patches. These cells with an unusual phenotype are present in fairly large numbers in the Peyer's patch and may play a key role in IgA B cell differentiation.

Dr. James and his group have continued their study of Leu-8 protein marker and its function in the lymphocytes of the mucosal immune system. Leu-8+ cells are present in mesenteric lymph nodes but not in lamina propria of the bowel. They have been shown to have suppressor activity in controlling immunologic function. Working with Brian Seed Leu-8 antigen has been successfully cloned and sequenced. Activation of lymphocytes was associated with a decline in Leu-8 mRNA and with the loss of Leu antigen from the cell surface. The sequence of the Leu-8 molecule suggests that it may act in a homing function allowing cells to travel to the sites in the body where they function. Thus, the molecule has been identified as an important and unusual surface molecule and its functions can now be determined. The capacity of mucosal lymphocytes to produce various lymphokines has also been determined using Northern Blot analysis of RNA from mitogen activated cells from various locations. The types of cytokines and interferons expressed was determined. The differences between lymphocytes obtained from the lamina propria of the bowel and mesenteric lymph nodes has been explored and striking differences have been shown; eg some of these cells are making IL-4 and others are making IL-2 and INF-gamma. IL-2 and INF-gamma enhance immunoglobulin production and IL-4 decreases immunoglobulin production.

Dr. Strober and Dr. James' data suggest that T cells in inductive sites such as the mesenteric lymph node are primarily naive cells that are less capable of producing lymphokines that cause terminal B cell differentiation. This population is able to expand under the influence of certain cytokines like IL-2 and IL-4. The cells in the lamina propria are primarily differentiated memory cells that have limited capacity to respond to IL-4 but can respond to other recall signals.

ALLERGY GROUP:

ALLERGIC DISEASES SECTION

The Allergy group has also continued to make important clinical and basic scientific advances in studies of IgE receptor function and the mechanism of its action in triggering basophils. Major new information is being obtained on the role of phosphatidylinositides in mast cell and basophil triggering. The system that the group has worked out allows for detailed biochemical studies of IgE triggering of cells and its biochemical consequences. Studies of calcium flux and the role of serine proteases will allow us to understand the mechanisms by which allergy causing immunoglobulin has its affect.

Major studies have advanced our knowledge of nasal response to external provocation. Histamine was found to cause increased vascular permeability mediated via H1 receptors. Methacholine on the other hand caused selective granular secretion that was inhibited by atropine. The usefulness of atropine in rhinitis caused by hot spicy food was demonstrated. The role of neuropeptides in nasal mucosal function is under study and the distribution of receptors for these peptides has now been mapped in the nose. The group's studies indicate that mast cells are not activated by endogenous neuropeptide release and that neuropeptide release does not play a major role in allergic responses. Patients with recurrent sinusitis were found to have a defect in response to cholinergic stimuli raising the possibility that such a defect may predispose to sinusitis. Mucous secretion has also been under study by this group for many years. Glucocorticoids have been shown by the group to decrease mucous glycoprotein via a lipocortin-mediated effect. The neuropeptide substance P has been shown to cause mucous glycoprotein secretion as does platelet activating factor. The group has developed a new assay for histamine which they hope will be widely used and replace other available methodology. The group also showed this year that Bronchopulmonary dysplasia can be separated from hyaline membrane disease by the relative absence of lactoferin and lysozyme in tracheal aspirates. This may give important clinical insights into this difficult disease of childhood.

MAST CELL PHYSIOLOGY SECTION

Dr. Metcalfe's group continues its detailed studies of mast cell growth and differentiation both at the basic and clinical levels. It was shown that dexamethasone inhibits many aspects of mast cell synthesis in a mouse cell line. It was also found that cultured mouse mast cells and human basophils adhere to laminin, a cell matrix protein. Adherence requires activation of the cell but not histamine release and messenger RNA for a laminin receptor was detected within the cells. This may provide an important clue to how mast cells travel and attach to matrix, thereby improving an understanding of their function.

Patients with systemic mastocytosis have been under study by the group for a period of time. The usefulness of a glucocorticoid with important gastrointestinal activity, Tixocortol Pivalate has been explored. It may prove to be of value in the treatment of inflammatory disease of the bowel and in treatment of mast cell infiltrates in the bowel. Effective therapy of mast cell induced bowel disorders is not available and this may provide an important advance. Splenectomy was found to lengthen survival, interestingly, in a group of patients with severe mastocytosis progressing to an aggressive malignant-like disease and should be considered in patients who do not have indolent mastocytosis. Other therapeutic agents are being studied for their clinical effect.

In collaborative studies with Dr. P.V. Subba Rao at the Indian Institute of Science the group has characterized some of the important antigens in shrimp and are now studying the immune response to these important antigens. Using ideotype specific antibodies they have been able to induce immune responses to shrimp antigens in the absence of actual antigen administration. The group has now proven that aspartame immunity is very infrequent and probably not clinically significant.

Honors and Awards

The present Chairman of the Board in Allergy/Immunology is Dr. Warren Strober. Last year's Chairman was Dr. Michael Kaliner. The Chairman next year will be Dr. Robert Rich, a graduate of our program. Similarly, several years ago the Chairman of the Board of Internal Medicine was Dr. Richard Root a graduate of our program. The current Chairman has been Dr. Sheldon Wolff a graduate of our program and former Clinical Director and Dr. Harry Kimball a graduate of our program will be the next Chairman of the Board in Internal Medicine.

Allergic Diseases Section

Michael Kaliner, M.D., Head, Allergic Diseases Section, LCI, NIAID

Asked to join the Executive Committee of the International Association of Allergy and Clinical Immunology

Joined the Executive Committee of the American Academy of Allergy and Immunology

Joined the Executive Committee of the Allergy and Asthma Network

Named Editor of the NIH Observer

Invited to deliver the "Roy Patterson" lecture at the American Academy of Allergy and Immunology Meeting

Invited to deliver the "Jaros" lecture at the American College of Allergy Meeting

Martha White, M.D.

Won the "Merrell Dow Scholar in Allergy Award" as the outstanding young allergy investigator in the United States. This award provides $50,000 towards salary support.

Bacterial Diseases Section

John I. Gallin, M.D., Director, DIR, NIAID

Meritorious Service Medal, United States Public Health Service, May 1988

Harry L. Malech, M.D., Head, Bacterial Diseases Section, LCI, NIAID

Director's Award, National Institutes of Health, June 1989.

Clinical Immunology Section

Michael M. Frank, M.D., Chief, Laboratory of Clinical Investigation, NIAID

Continues as a Deputy Editor, Journal of Immunology

Sits on the Board that presents the Burroughs Wellcome Grant for $300,000 for research in Immunopharmacology and will be Chairman of that Board next year.

Invited speaker at the International Immunology Society and is a featured speaker at the Southern Medical Society.

(Clinical Immunology cont.)

Carl Hammer, Ph.D.

Received the NIAID Director's Award

Clinical Mycology Section

John E. Bennett, M.D., Head, Clinical Mycology Section, LCI/NIAID

Serves on the Subspeciality Board in Infectious Disease, American Board of Internal Medicine

Kyung Kwong-Chung, Ph.D.

Keynote Speaker for the Medical Mycology Division of the American Society for Microbiology

Keynote Speaker for the Mycological Society of America

Mast Cell Physiology Section

Dean Metcalfe, M.D., Head, Mast Cell Physiology Section, LCI, NIAID

Invited to give the Paul F. de Gara Lecture in Allergy and Immunology, Cornell Medical Center, 1988

Chairman of the AAAI Interest Section on Foods and Drugs, 1988-89

John M. Sheldon Memorial Lecture, AAAI Meetings, San Antonio, Texas, 1989

Appointed to the Editorial Boards of the Journal of Immunology, the Journal of Clinical Immunology, and Clinical Reviews in Allergy

Appointed to the Chairmanship of the Allergy/Immunology Committee for MKSAP IX for American College of Physicians

Medical Virology Section

Kenneth D. Croen, M.D.

ICAAC Young Investigators Award, October, 1988

Mucosal Immunity Section

Warren Strober, M.D., Head Mucosal Immunity Section, LCI/NIAID

Elected Chairman of the Speciality Board in Allergy/Immunology

Deputy Editor, Journal of Immunology

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 0043-**24**-LCI

PERIOD COVERED

Oct. 1, 1988 to Sept. 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John E. Bennett, Head, Clinical Mycology Section	LCI/NIAID
Others:	Edward Mcmanus, Medical Staff Fellow	LCI/NIAID
	Virginia Kan, Guest Worker	LCI/NIAID
	François Dromer, Visiting Fellow	LCI/NIAID
	John Rex, Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)* 1. Pulmonary Disease Division (Dr. Henry Yaeger) Georgetown Univ. Hospital, Washington, D.C./2. Dept. of Medicine, Tufts University School of Med., Boston, MA (Dr. Jeffrey Gelfand)/ 3. Cedars-Sinai Medical Center, Los Angeles, California (John Chia, M.D.)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

TOTAL MAN-YEARS | PROFESSIONAL | OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies have continued on the lectin-like receptor by which human pulmonary alveolar macrophages bind Aspergillus fumigatus conidia. A trypsin-sensitive site on the macrophage was found to recognize a ß1-3 glucan configuration on conidia. Macrophage CR3 was determined not to be involved in conidial binding.

Release of TNFα and IL-1 was found to correlate with pyrogenicity in lots of desoxycholate amphotericin B.

Iodide therapy of normal volunteers was found not to improve killing of Sporothrix schenckii by neutrophils or monocytes in vitro.

Normal neutrophils and neutrophils deficient in oxidative fungicidal mechanisms were found to cooperate in damaging Aspergillus hyphae in vitro. Normal neutrophils provided hydrogen peroxide which could be used by defective neutrophils.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00045-21 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael M. Frank, Chief, LCI/NIAID

Others: Louis Fries, Senior Staff, NIAID
Carl Hammer, Senior Staff, NIAID
Tom Waytes, Medical Staff Fellow, NIAID
Paul Langlois, IRTA
Yannick Pilatte, Visiting Fellow, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 2.7	PROFESSIONAL: 2.2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have explored the effect of gamma interferon on the clinical course of Hereditary Angioedema. A limited number of patients with Hereditary Angioedema have disease that responds to no therapy. These people present a tremendous management problem because there is no effective way to manage their life threatening disease. Monocytes obtained from the peripheral blood have been noted to produce C1 inhibitor, the protein that is missing in Hereditary Angioedema, and it has been noted in in-vitro studies that gamma interferon can induce monocytes to increase their synthesis of this protein. We therefore have initiated a clinical trial of gamma interferon therapy to determine whether we could induce either a biochemical or therapeutic response with this agent. Five patients were treated with gamma interferon after a period of time devoted to obtaining baseline studies. All responded with an increase in their levels of C1 Inhibitor and all reported that they felt somewhat improved. However, none experienced total clinical remission of their underlying disease. Moreover, a wide range of toxic side effects of the drug were noted. The most severe was headache but neutropenia was also noted. In no case did the C1 Inhibitor return to the range of normal. In 3/5 patients the clinical trial had to be halted because of severe toxicity. Although this agent is helpful in control of this disease it can be considered no more than an adjunct agent at this time.

We have previously shown that purified C1 Inhibitor can be used to successfully terminate attacks of Hereditary Angioedema. This information has not proven useful clinically because this serum protein is difficult to purify and there is no commercial source of this protein available in this country. We have developed a new method for the purification that is quick, simple and yields the most highly active protein yet available. This method lends itself to commercial preparation of this serum protein. It is hoped that this new method will allow preparation of this protein on a commercial scale for clinical use.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00047-20 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Head, Clinical Parasitology Section	LCI/LPD, NIAID
	T.E. Nash	Senior Investigator	LCI/LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID
		Senior Investigator	LCI, NIAID

Others: **See next page**

COOPERATING UNITS *(if any)*

See next page

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of these studies is to increase understanding of the pathogenesis of parasitic diseases and to improve their diagnostic, therapeutic and preventative measures. In addition to studies at NIH, clinical investigation of patients has been carried out in the endemic areas of Guatemala (onchocerciasis), India and Brazil (filariasis), Zaire and Jamaica (strongyloidiasis), Honduras (cysticercosis and leishmaniasis) and Panama (leishmaniasis).

Such studies have clearly defined an association between HTLV-1 infection and strongyloidiasis, but there seems to be no similar relationship with HIV infections.

Laboratory studies have permitted definition of the regulatory and pathogenetic mechanisms underlying the IgE, IgG4, and eosinophil responses to helminth infection, as well as the identification of molecules responsible for antigenic variation in giardiasis likely involved in protective immunity in bancroftian filariasis, onchocerciasis and strongyloidiasis.

Therapeutic trials of ivermectin for bancroftian filariasis have shown it to be effective and safe in clearing microfilaremia and, because of its single-oral-dose mode of therapy, to be considerably more practical for use in control programs than the older antifilarial drug, diethylcarbamazine.

Other Professional Personnel

T. Nutman	Senior Investigator	LPD, NIAID
L. Kramer	Special Volunteer	LPD, NIAID
A. Cheever	Assistant Chief	LPD, NIAID
A. Limaye	Howard Hughes Fellow	LPD, NIAID
T. Fleischer	Chief, Clin Immunol Service	CPD, Clinical Center
D. Sacks	Staff Fellow	LPD, NIAID
P. Melby	NRSA Fellow	LPD, NIAID
D. Freedman	Medical Staff Fellow	LPD, NIAID
B. Gottstein	Visiting Scientist	LPD, NIAID
N. Raghavan	Visiting Fellow	LPD, NIAID
R. Adam	Medical Staff Fellow	LPD, NIAID
C. Brown	Medical Staff Fellow	LIR, NIAID
A. Klion	Medical Staff Fellow	LPD, NIAID
M. Mowatt	Staff Fellow	LPD, NIAID
E. Lobos	Visiting Fellow	LPD, NIAID
C. King	Medical Staff Fellow	LPD, NIAID
R. Nussenblatt	Chief	CB, NEI
P. Brindley	Special Volunteer	LPD, NIAID
M. Ballard	Medical Staff Fellow	LPD, NIAID
S. Greenberg	Medical Staff Fellow	MB, NCI
W. Blattner	Chief, Viral Epidemiology	EEB, NCI

Cooperating Units

University of California at San Francisco (J. McKerrow); Peace Corps Medical Office, Washington, DC (M. Mulligan); Department of Health, Guatemala (G. Zea-Flores); University of Michigan (J. Williams); Indian Council of Medical Research Tuberculosis Research Center, Madras, India (S.P. Tripathy, R. Prabhakar, V. Kumaraswami, and V. Vijayan); Madras Medical College, Madras, India (K. Vijayasekaran); Instituto de Salubriadad y Enfermadades Tropicales, Mexico City, Mexico (O. Velasco); SEMA, Inc., Rockville, MD (G. Phillips); Merck, Sharpe Dohme, Rahway, NJ (D. Neu, B. White-Guay); University of Maryland, Baltimore, MD (M. Levine; D.A. Harrington, G.A. Losonsky, S. Giannnini, S. Savarino); Ministry of Health, Tegucigalpa, Honduras (C. Ponce); Dept. of Biology, Youngstown University, Ohio (R. Kreutzer); Yale University School of Public Health, Dept. Epidemiology, New Haven, CT (D. McMahon-Pratt); Uniformed Services University of the Health Sciences (R. Lal); New England Biolabs, Beverly, MA (F. Perler, L. McReynolds, C. Maina); Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil (A. Continho, G. Dreyer); Aga Khan Medical School, Karachi, Pakistan (R. Hussain); E.R. Squibb and Sons, Princeton, NJ (A. Lentnek); University of the West Indies, Jamaica (P. Morgan, R. Robinson, S. Terry); Project SIDA, Kinshasa, Zaire (J. Perriens); Georgetown University, Washington DC (W.T. London); Anna University, Madras, India (K. Jayaraman); Thanjavur Medical School, Thanjavur, India (S. Jamal); U. South Florida, Tampa, FL (A. Vincent).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00048-19 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)
PI: Michael M. Frank, Chief, LCI/NIAID
Others: C. Hammer, Senior Investigator, LCI/NIAID
L. Fries, Medical Officer (Research), LCI/NIAID
M. Marques, Visiting Fellow, LCI/NIAID
P. Langlois, IRTA
M. Basta, Visiting Fellow, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3	PROFESSIONAL: 3	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of antibody and complement in the clearance of erythrocytes from the circulation have continued at a much reduced pace because of other activities related to presented projects. Specific questions include the reasons for the difference between effects of IgG and IgM antibodies. IgG antibody causes splenic sequestration at low doses, IgM antibody causes hepatic sequestration. In both cases, complement is required for this effect. A second question is the complement receptors involved in such clearance mechanisms.

An unexpected finding this year is that serum C1 inhibitor is the controlling protein that determines the extent of complement activation on a target. The interactions of this protein with the C1 on a target have been studied with regard to consequences of this activation process.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-0057-16-LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Basic Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

K.J. Kwon-Chung	Research Microbiologist	LCI/CMS/NIAID
R. Kelly	Senior Staff Fellow	LCI/CMS/NIAID
A. Varma	Microbiologist	LCI/CMS/NIAID
W.L. Whelan	Visiting Scientist	LI/CIS/NIDR

COOPERATING UNITS (if any)

NIDR

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Topics of current studies include 1) biotype patterns of Candida albicans and Cryptococcus neoformans from AIDS and Non-AIDS patients; 2) genetic differences between avirulent and virulent isolates of Candida stellatoidea; 3) cloning of an α-glucosidase gene from C. albicans. We have compared the biotypes of C. albicans isolates from 23 AIDS patients suffering from oral and/or esophageal candidiasis with isolates from oral cavities of 17 healthy volunteers. The isolates revealed no significant difference in distribution among 12 phenotypic classes defined by genetically determined resistance to 5-Fluorocytosine, heterozygosity at genes which determine synthesis of aminoacids, purines, pyrimidines, and dimorphism of an Eco RI restriction fragment of DNA which encodes ribosomal RNA. Restriction fragment fingerprints of cellular DNA failed to distinguish between oral and esophageal isolate from each AIDS patient tested. The Eco RI restriction fragments remained the same in isolates taken from one patient at three times in a five month period. These results indicated that in each patient a single C. albicans strain was responsible for infection at both oral and esophageal sites for a prolonged period of time. Isolates of Cryptococcus neoformans from AIDS and Non-AIDS patients of Southern California were compared with isolates of Zairean AIDS patients. The genetic diversity seen among Southern Californian AIDS isolates was similar to that of Non-AIDS isolates. The isolates from Zairean AIDS patients showed more genetic diversity than those of Californian isolates. The avirulent isolates of Candida stellatoidea designated as type I were differentiated from virulent isolates of C. stellatoidea type II by their electrophoretic karyotype, mitochondrial DNA restriction patterns, and midrepeat sequence of nuclear DNA. The karyotype and major bands of the midrepeat sequence of type II were inditinguishable from those of the reference C. albicans. Mt DNA restriction patterns of type II isolates were either the same as type I C. stellatoidea, or the same as C. albicans depending on the enzyme-probe combinations used. All type I isolates were serotype B, while type II isolates belonged to serotype A. Genetic analysis by complementation tests showed that the sucrose-negative phenotypes of type I and type II are due to different mutations. A Candida albicans α-glucosidase gene (2.3 kb in size) which complemented the sucrose utilization defect of type II C. stellatoidea upon transformation was cloned.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 0058-15 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Chemotherapy of Herpesvirus Infections in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
	J.Ostrove	Senior Staff Fellow	LCI,NIAID
	K.Croen	Medical Staff Fellow	LCI,NIAID
	A.Freifeld	Guest Researcher	LCI,NIAID
	R.Holman	Medical Staff Fellow	LCI,NIAID
	P.Krause	Medical Staff Fellow	LCI,NIAID
	J.Dale	Clinical Research Nurse	LCI,NIAID
	S.Nagpal	Visiting Fellow	LCI,NIAID
	M.Levin	Guest Researcher	LCI,NIAID
	L.de Armas	Guest Worker	HHMI

COOPERATING UNITS *(if any)*

J.Rooney, Senior Staff Fellow,LOM,NIDR; A.Notkins, Chief,LOM,NIDR; J.Hilliard (Southwest Fndn.for Biomed.Res.); J.Southers,RVR; Y.Bryson (UCLA)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of the project are to characterize the pathogenesis, natural history and therapy of herpes simplex virus and varicella-zoster virus infections. Our clinical emphasis has been on genital herpes in normal and immune-impaired patients. Analysis of six years of suppressive acyclovir in patients with frequently recurring herpes have shown the drug to remain effective, to be well-tolerated, and to not induce drug resistance. We continue to study the ability of ultraviolet light and other physical and chemical agents to induce recurrent herpes simplex infections in humans. A placebo-controlled trial of acyclovir for blocking u-v induced reactivation is nearly complete. We also initiated a placebo-controlled trial of acyclovir for suppression of frequent, spontaneously recurring oral herpes.

The major thrust of our laboratory effort in this project has been the analysis of herpes simplex latency in human ganglia. We demonstrated HSV-1 latency-associated RNAs in trigeminal ganglia and HSV-2 RNAs in sacral ganglia. During the past year primer extension analyses, sequencing and transfections of CAT constructs were used to further define the structure, kinetics, and regulation of these latency-associated RNAs. A putative "variant" promoter has been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00154-14 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Events in Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID
	Mary E. Revenis, M.D.	Guest Researcher	LCI/NIAID
	Marek L. Kowalski, M.D.	Fogarty Visiting Fellow	LCI/NIAID
	Alain Didier, M.D.	Special Volunteer	LCI/NIAID
	Yasushi Igarashi, M.D.	Fogarty Visiting Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*
Julie Goth, Ph.D., Georgetown University (Contract # N0-1-A1-22665)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0

CHECK APPROPRIATE BOX(ES)
☐x (a) Human subjects ☐x (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This broad-based project explores allergic mechanisms at a number of levels. Major observations uncovered include developing a new, sensitive assay for histamine which is simple enough for any laboratory to perform easily. This assay will certainly become the standard, replacing an assay we developed several years ago. Antidromic nerve stimulation is an excellent model for exploring the role that endogenous neuropeptides play in disease. We have characterized neurogenic inflammation in rat cutaneous and pulmonary models and have searched for a possible connection to mast cell activation. Our studies indicate that mast cells are not activated by endogenous neuropeptide release and that neuropeptide release does not play a major role in allergic responses. We have contrasted neutrophil derived histamine releasing factor with a number of other mast cell activators, and have found HRA-N to be unique in regards to the spectrum of its activity, the kinetics of mast cell activation, and its biologic characterization. Bronchopulmonary dysplasia can be discriminated from hyaline membrane disease by the relative absence of lactoferrin and lysozyme in tracheal aspirates. This finding suggests that the relative absence of serous cell products predisposes to bronchopulmonary dysplasia (BPD). Bronchopulmonary dysplasia affects 40% of children weighing less than 2 kg. It is estimated that each child with BPD costs society $300,000/yr for hospital-related expenses. Our finding of reduced serous cell products in this disease provides the first insight into a pathologic process which may be involved. Moreover, these findings provide a new insight into possible therapeutic interventions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-14 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phagocytic Cell Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Scientific Director, DIR,	NIAID
Others:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
	Karen Lomax, M.D.	Senior Staff Fellow	LCI/NIAID
	Daniel Rotrosen, M.D.	Senior Staff Fellow	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID
	Hiroyuki Nunoi, M.D.	Visiting Associate	LCI/NIAID
	Stuart Abramson, M.D.,Ph.D.	Med. Staff Fellow	LCI/NIAID
	Richard Kenney, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)* D Melnick, DIR/NIAID; J Rex and J Bennett, LCI/NIAID; HC Lane and AS Fauci; B Volpp, W Nauseef, and R Clark Dept. Medicine, Univ. Iowa, Iowa City, IA; R Lehrer, Dept. Med., Univ. California Los Angeles, Los Angeles, CA.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the genetic basis of inherited diseases affecting neutrophil function and associated with recurrent infections, and diagnosis and treatment of such disorders. Chronic Granulomatous Diseases of Childhood (CGD) are a group of diseases with common phenotype including absent H2O2 production by phagocytes, recurrent infections, and granuloma formation. The X-linked form results from any of a number of defects in the gene coding for the 91 kDa subunit of cytochrome b558. We have documented the prevalence of the four distinct genetic forms of CGD. Using cDNA probes to 47 kD and 65 kD neutrophil cytosolic factors missing from two forms of CGD, we showed that RNA transcripts for the missing protein factors are present. We find that neutrophils from a subset of X-linked CGD patients forming granulomas, tend to express some cytochrome b and retain residual superoxide produc ing capability. Sterile granulomas in CGD patients may be associated with neurologic dysfunction, urinary tract obstruction, colitis, pyloric dysfunction, and poor healing of surgical wounds. These granulomas respond to low dose corticosteroids. We document fungal infection in CGD patients with unusual fungal species (Aspergillus nidulans, brown molds, and trichosporon species). Appropriate diagnosis and therapy requires sugical biopsy and excision of devitalized infected tissues. Using an in vitro assay of fungal killing by neutrophils, we have documented that a small number of normal neutrophils act synergistically with a larger number of CGD neutrophils to kill aspergillus hyphae providing a rationale for use of granulocyte transfusions in infected CGD patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-08 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Beth Friedman, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*

Clinical Pathology Department, NIH Clinical Center (Dr. William Travis)
CHB, NHLBI (Dr. Neal Young)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	1.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tixocortol Pivalate, a steroid without significant adrenal-pituitary axis suppression when administered orally, was given to four patients with systemic mastocytosis and severe gastrointestinal disease. All patients experienced a decrease in abdominal pain and stool frequency which paralleled improvement in malabsorption as determined by laboratory parameters. Tixocortol Pivalate may prove to be of value in the therapy of inflammatory diseases of the upper gastrointestinal tract that require prolonged steroid administration.

The records of 26 patients with systemic mast cell disease were reviewed to determine the role of splenectomy in the management of this disease. Splenectomy had been performed in five of nine patients with aggressive disease associated with a hematologic disorder or a lymphoma-like course. Length of survival was improved with splenectomy with patients better able to tolerate chemotherapy. Thus, while splenectomy is of no value in the management of indolent mastocytosis, it should be considered in patients with aggressive systemic mast cell disease.

Fifteen adults with mastocytosis were entered into a double-blind protocol comparing azelastine, a drug with mast cell stabilizing properties, with chlorpheniramine, an H1 antihistamine. Agents were compared with symptom scores, by analyzing plasma and urine histamine levels, and by comparing suppression of skin test responses to degranulating agents. The trial is completed and data analysis is underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

Z01 AI 00271-08 LCI

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
October 1, 1988 to September 30 , 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Purification and Characterization of Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: Carl H. Hammer, Senior Investigator, LCI/NIAID
Others: Michael M. Frank, Chief, LCI/NIAID
Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID
Yannick Pilatte, Visiting Fellow, LCI/NIAID
Paul Langlois, IRTA , LCI/NIAID
Milan Basta, Visiting Fellow, LCI/NIAID
Louis F. Fries, Medical Officer, LCI/NIAID
Lois Renfer, Chemist, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.4	1.9	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have recently reported the isolation and partial characterization of a new plasma sialoglycoprotein with a molecular weight of 120 kDa (sgp120). This protein has a number a properties similar to that of the second component of human complement (C2) and they are coisolated on C4b-Sepharose; the following differences have been noted. Sgp120 is present in plasma at 15 x the concentration of C2. These proteins do not cross immunoprecipitate with polyclonal antisera. They are activated and cleaved by distinct mediator pathways: sgp120 by the contact-activation system (CAS) and C2 by the classical complement pathway (CCP). The proteins have unique N-terminal amino acid sequences and a C-terminal stretch of 50 residues within sgp120 as determined by cDNA cloning and sequencing is unrecognized in any of 6000 screened sequences including C2, kallikrein and HMW kininogen. We have developed new methodology to prepare sgp120. Sgp120 cleavage was shown to be mediated by kallikrein in the contact-activation system resulting in the generation of distinct cleavage fragments. Initial proteolysis produces cleavage at 85 kDa from the N-terminal producing a 35 kDa fragment. Subsequent cleavage of the 85 kDa fragment at 60 kDa from the N-terminal produces a 25 kDa fragment. The ordering of the peptides was deduced from the N-terminal amino acid analyses of the respective components. We have shown that sgp120 is a sialoglycoprotein containing about 6% sialic acid and the same amount of N-linked carbohydrates as C2. However, sgp120 also contains 0-linked carbohydrates. Monoclonal antibodies which specifically bind to sgp120 were produced. One of these was produced from purified sgp120 while the other was produced by immunization with purified C2. This latter monoclonal was found to be cross-reactive with sgp120 during the process of subcloning of the original C2 antibody. During these studies a fast and efficient, high yield, method for the purification of C1-inhibitor , the major regulatory protein of the CCP and CAS, was developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00272-07 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Host Defense Against Pneumococcal Bacteremia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael M. Frank, Chief, LCI/NIAID

Others: David Bobak, Medical Staff Fellow, LCI/NIAID
Ronald Washburn, Medical Staff Fellow, LCI/NIAID
Andrea Tenner, Scientist One, Dept. of Biochem., Amer. Red Cross Biomedical Research and Development, Rockville, Maryland

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00275-08 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Complement Receptor and C3 Mediated Opsonization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thelma Gaither, Research Biologist

Others: Michael M. Frank, M.D., Clinical Director
Yannick Pilatte, Ph.D., Visiting Fellow
Marva Moxey-Mims, M.D., Guest Research
Evelyn Lin, Chemist
Glenn Kolluri, Laboratory Worker

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)* We reported previously the PMN oxidative burst reduces phagocytosis as evidenced by elevated phagocytosis by PMN of CGD patients and by enhanced phagocytosis in the presence of inhibitors of MPO and HOCl. This suggested that the oxidant, HOCl (formed by the MPO, H_2O_2, Cl reaction), or its derivatives, regulates Fc receptor (FcR) expression or function. The effect of HOCl was examined by treating PMN with HOCl and measuring binding of ^{125}I-labeled monoclonal antibodies (MoAb) against various membrane receptors. HOCl reduced by $\geq$50% the binding of two MoAb's against the IgG-binding site of FcRII and that of one MoAb against the IgG-binding site of FcRIII. Notably, HOCl had little effect on binding a MoAb against a non-IgG binding site on FcRIII. It also caused reduction in binding of two MoAb's against the iC3b-binding site of CR3 and also that of anti-β2m, but caused little change in binding of MoAb against the C3b-binding site (anti-CR1) or anti-HLe-1. A reduction in the activity of the functional binding site of the FcR's, but not that of CR1, was also demonstrated by 50% loss in EIgG rosetting and no loss in that of EC3b by HOCl-treated PMN. HOCl had a similar effect on binding of MoAb's to immobilized PMN extracts as shown by immuno-dot blotting. FcR and CR3 down-regulation by oxidative products generated during phagocytosis may represent an important internal control mechanism that turns off further phagocytosis by PMN that have ingested their "fill" of invading organisms. We have reported that phagocytosis by PMN from patients with acute bacterial infections is enhanced over normals. Since several mediators are involved in the inflammatory and infectious process, we studied the effects of purified mediators on phagocytosis by PMN of normal donors. IL-1 and TNF each caused a significant increase in the ingestion of EIgG by adherent PMN, whereas IL-2 had no significant effect. In combination, IL-1 and TNF showed an additive effect, suggesting that they act on different subpopulations of PMN to mediate enhanced phagocytosis. The effect of these mediators was not due to an increase in the expression of surface FcRI, II or III. Notably, IL2 blocked TNF-mediated enhancement of phagocytosis, but not that of IL1. This finding may have important implications physiologically, since it is known that patients on IL-2 therapy are highly susceptible to infections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00278-08 LCI

PERIOD COVERED
October 1, 1988 to September 30 , 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Components of the Complement Cascade

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Carl H. Hammer, Senior Investigator, LCI/NIAID
Others: Michael M. Frank, Chief, LCI/NIAID
Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID
Paul F.Langlois, IRTA , LCI/NIAID
Yannick Pilatte, Visiting Fellow, LCI/NIAID
Gilda Linton, Medical Technologist, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.6	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have examined the functional properties of a newly recognized sialoglycoprotein of 120 kDa (sgp120) which has a normal plasma concentration of 325 µg/ml. This protein coelutes from C4b-Sepharose with the second component of complement, C2, and has a number of properties in common with this member of the classical complement pathway (CCP). We have clearly shown the unique nature of sgp120 and have characterized it as a new and distinct plasma protein which participates in both the CCP as well as the contact-activation system (CAS), see accompanying project # Z01 AI 00271-06. Studies with purified complement components showed that low levels of sgp120 (4-7 µg) effectively inhibit the formation of CCP convertase sites > 90%. This inhibition was shown to be dose responsive for C1, C4, C2, and C3 site formation. Use of radiolabeled sgp120 showed its specific binding to C4b-coated, antibody sensitized, sheep erythrocytes. Binding was both dose responsive and saturable showing specificity for C4b. Binding was competitively inhibited by both cold sgp120 as well as C2. These data support sgp120 as a new complement regulatory protein.

We have also studied sgp120 and its cleavage products for the presence of biologic activity as assessed by the ability to cause capillary leakage in the skin of a guinea pig and contraction of an isolated guinea pig ileum. Sgp120 fragments, produced by the serine protease kallikrein, possessed both the above activities; the intact protein was inactive in these assays.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-08 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID

COOPERATING UNITS *(if any)*

James H. Shelhamer, M.D., Carolea Logun, and Jens D. Lundgren, Critical Care Medicine, Clinical Center

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion.

Corticosteroids inhibit MGP release by lowering baseline secretion. Analysis of corticosteroid treated airways reveals a close correlation between lipocortin generation and MGP production. Pulmonary inflammation with neutrophils is often associated with mucus production. Lysates of human neutrophils as well as supernatants from activated neutrophils cause airways to release MGP; this activity is not due to elastase, and identification of the mucus secretagogue is under study. Corticosteroids reduce the goblet cell hyperplasia caused by exposure to neutrophils.

Substance P is found in close association with feline submucous glands. Substance P causes MGP secretion, and the activity of substance P is markedly increased by protection from degradation by proteases with thiorphan.

Platelet activating factor (PAF) activates feline airways to secrete MGP, and this action requires the formation of arachinoids and is inhibitable by CCS.

GRP is a stimulant of feline airway submucosal glands.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00354 07 LCI

PERIOD COVERED October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS *(if any)*

David Camerini, Department of Genetics, Harvard Medical School
Brian Seed, Department of Genetics, Harvard Medical School

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously we have shown that there is a striking difference in the distribution of Leu-8 positive cells in the inductive and effector compartments of the mucosal immune system; Leu-8+cells predominate in the mesenteric lymph nodes and are absent in the lamina propria. Since Leu-8+ T cells have suppressor activity which is enhanced by cross-linking of their Leu-8 antigen, this observation has important implications for the function of the mucosal immune system. To further define the nature of the Leu-8 antigen we carried out a series of biochemical, molecular and functional studies. Immunoprecipitation of surface-labeled T cells showed that the Leu-8 antigen is a single chain 80 kDa molecule which is heavily glycosylated, there being two closely related core structures with apparent MW of 52 and 54 kDa. Western blotting of membrane extracts showed the presence of a single chain molecule which rapidly disappears from the cell membrane within hours of cell activation. Using a monoclonal antibody panning method, two cDNA clones expressed transiently on the cell membrane of COS cells were obtained from a human lymphocyte cDNA library. These two clones both hybridized with lymphocyte RNA of 1.9 and 2.3 kb. Activation of lymphocytes was associated with a decline in Leu-8 mRNA, which occurred much more slowly than the rapid disappearance of the Leu-8 antigen from cell membranes, indicating that the decline in Leu-8 protein on activated lymphocytes is the result of both transcriptional and post-transcriptional regulatory events. The nucleotide sequence of the putative Leu-8 clones revealed two similar sequences differing in their predicted transmembrane regions. The sequences had close homology with the reciently sequenced murine MEL-14 homing receptor, a member of a newly described family of molecules (dubbed LEC-CAMs) which have an external lectin-like domain, an EGF like domain, and tandem repeats of complement receptor domains. Thus, we have identified the Leu-8 molecule as a member of a novel class of surface glycoproteins which may play an important role in lymphocyte migration and regulatory function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00355-06 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Defects in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Stephen P. James, Investigator, Mucosal Immuity Section, LCI/NIAID

COOPERATING UNITS *(if any)*

E. Anthony Jones, M.D., Chief, Section on Liver Diseases, NIDDK

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was inactive during this period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00356-07 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID
Other Dennis Y. Kunimoto, M.D., Fellow Alberta Heritage Foundation, LCI/NIAID
Gregory R. Harriman, M.D., Medical Staff FEllow, LCI/NIAID
Nils Y. Lycke, M.D., Visiting Scientist, LCI/NIAID
Richard p. Nordan, M.D., Senior Staff Fellow, LG/NCI

COOPERATING UNITS *(if any)*
Laboratory of Genetics, NCI

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During this period studies of the role of various cytokines, in particular, IL-5 and IL-6, in IgA B cell differentiation were conducted. These studies utilized recombinant IL-5 produced in a Baculovirus expression system in this laboratory. It was found using in vivo activated Peyer's patch B cells depleted of T cells, that whereas rIL-1 plus purified IL-6 support enhanced IgM and IgG (IgG3) synthesis, this combination has little effect on IgA synthesis. On the other hand, rIL-5 plus IL-6 formed a potent combination for the enhancement of IgA synthesis and did not effect IgM or IgG synthesis to a greater extent than IL-5 alone. These studies indicate for the first time that combinations of cytokines/lymphokines have Ig class-specific effects and explain the activity of certain subpopulations of T cells on IgA secretion in the mucosa.

In another series of studies the effect of cholera toxin (CT) or the B subunit of cholera toxin (CT-B) on LPS-induced B cell differentiation was determined. It was found using normal B cells that CT or CT-B induces the appearance of increased numbers of IgG and IgA and decreased numbers of IgM B cells in LPS-stimulated whole B cell cultures or cultures composed only of sIgM+ cells, but not in cultures containing sIgM- cells. Thus CT (and CT-B) were assumed to have an effect on B cell isotype switching. Corroborative results were obtained using a monoclonal sIgM+ B cell line, CH12.LX.

In a final series of studies we defined a new class of T cells that are localized in the germinal centers of Peyer's patches. These cells have the phenotype, CD3+, CD4+, Thy 1- and comprise 15-45% of Peyer's patch CD4+ T cell populations. Cells in this subset freshly obtained from Peyer's patches were found to have different activation requirements than Thy1+ T cell populations. In addition, Thy1- T cell lines (established with anti-CD3 stimulation plus IL-2) secreted 5-10 fold less IL-5 than comparable Thy1+ cell lines and were less able to support IgA synthesis. These Thy1- T cells may play a key role in IgA B cell differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00357-05 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
P.I. Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID
Other Stephen P. James, M.D., Investigator, Mucosal Immuity Section, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been inactive during this current period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00397-06 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Louis F. Fries, Medical Officer (Research), LCI/NIAID
Others: Michael M. Frank, Chief, LCI/NIAID
John Zurlo, Medical Staff Fellow, LCI/NIAID
Milan Basta, Visiting Fellow, LCI/NIAID
Paul Langlois, IRTA/LCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism of action of IVIG in preventing acute complement-dependent immune injury was examined in an in-vitro and two in-vivo models. Complement-dependent clearance of IgM-sensitized guinea pig erythrocytes was delayed in guinea pigs infused with human IVIG. The effect was found to be specific for IVIG; human albumin failed to alter the clearance of these targets. A single high dose of human IVIG (1800 mg/kg) prevented death in 70% of recipient guinea pigs subjected to otherwise lethal doses of rabbit anti-Forssman antiserum. Prior studies have shown that Forssman shock is due to complement mediated attack on target endothelium. Similar effect on survival was obtained by infusions of IVIG previously absorbed with packed guinea pig erythrocytes to remove anti guinea pig erythrocyte antibodies in the IVIG that might compete with the lytic rabbit antibodies. Purified guinea pig IgG was also effective. IVIG therapy did not affect the levels of C3 and C4. Thus, utilization of these components was not the mechanism of IVIG effect. C3 and C4 were activated to the same extent in both IVIG and control animals. In in-vitro studies, IVIG was able to profoundly inhibit C3 fragment deposition on guinea pig erythrocytes coated with IgG and IgM antibodies. Our results suggest that upon complement activation IVIG acts as a receptor for nascent complement components; by this mechanism IVIG markedly reduces the effectiveness of complement attack on target membranes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00429-05 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Studies on Nasal Responses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Gordon D. Raphael, M.D.	Medical Staff Fellow	LCI/NIAID
	Aleksander Z. Gawin, M.D.	Special Volunteer	LCI/NIAID
	James N. Baraniuk, M.D.	Special Volunteer	LCI/NIAID
	David B. Peden, M.D.	Medical Staff Fellow	LCI/NIAID
	Michiko Okayama, M.D.	Special Volunteer	LCI/NIAID
	Elizabeth V.M. Jeney, M.D.	Special Volunteer	LCI/NIAID

COOPERATING UNITS (*if any*)
James H. Shelhamer, M.D., Critical Care Medicine, Clinical Center, NIH

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.3	0.6

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The nasal mucosa is the first internal surface to encounter aeroallergens, airborne pathogens, and airborne toxins. Analysis of nasal responses will likely provide insights into normal host defense mechanisms. Provocation of human and guinea pig nasal mucosa stimulates secretion, and analysis of the components of these secretions has revealed new insights into the source of these secretions as well as possible mechanisms for the pharmacologic control of secretion. Histamine was found to primarily cause increased vascular permeability as reflected in relative enrichment of nasal secretions with albumin, nonsecretory IgA (NS-IgA), and IgG. These effects were inhibited with an H-1 antihistamine but not by atropine. Methacholine caused selective glandular secretion as reflected in increases in total protein, secretory IgA (sIgA), lysozyme, and lactoferrin. There was also an increase in albumin and IgG, but no relative enrichment of these proteins. Methacholine effects were inhibited by atropine. Hot, spicy foods may cause rhinorrhea in sensitive subjects. "Gustatory rhinitis" was found to involve an atropine-inhibitable muscarinic pathway as both symptoms and secretory responses could be prevented by pretreatment with atropine. The three division of innervation in the nasal mucosa were found to have discrete associations with neuropeptides: the sensory nerves contain GRP (a glandular stimulant), CGRP, SP, and NKA (which act to regulate venule permeability); the parasympathetic nerves contain VIP, and sympathetic nerves contain NPY. Bradykinin was found to stimulate the vascular bed but not the glands directly; thus, bradykinin may participate in the vascular permeability seen in colds. Patients with recurrent sinusitis were found to have a singular defect in nasal secretory responses to cholinergic stimulation. Therefore, these patients may be predisposed to recurrent infections by the absence of specific and nonspecific host defense molecules.

Other Professional Personnel (cont.):

Phyllis R. Brayton	Microbiologist	LCI/NIAID
Joaquim Mullol, M.D.	Special Volunteer	LCI/NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00436-05 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Varicella Zoster Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
	J. Ostrove	Senior Staff Fellow	LCI,NIAID
	P. Krause	Medical Staff Fellow	LCI,NIAID
	K. Croen	Medical Staff Fellow	LCI,NIAID
	R.Holman	Medical Staff Fellow	LCI,NIAID
	L. Safranek	Medical Staff Fellow	LCI,NIAID
	S. Nagpal	Visiting Fellow	LCI,NIAID
	M.Levin	Guest Researcher	LCI,NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of CLinical Investigation

SECTION
Medical VIrology SEction

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project constitutes one of the two major laboratory research focuses of this section. The goals are to identify, map and characterize varicella-zoster virus genes and proteins expressed during active, latent, or productive infections, and to define the temporal sequence and control of gene expression. To accomplish these ends we use an extensive array of molecular biological techniques. To date we have mapped the viral genome and most of its transcripts. We have identified and carefully mapped a number of important viral gene products. Last year we proved that VZV remains latent in satellite cells of human sensory nerve ganglia and that some, but not all, VZV genes are expressed during latency. This year we have subcloned VZV genome fragments so that individual genes can be assayed for expression of transcripts during latency. Preliminary data indicate expression of only 4 to 13 VZV genes in latently infected human ganglia. Our findings with regard to VZV latency suggest a model by which the pathogenetic differences between zoster and recurrent HSV infections may be explained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00432 05 LCI

PERIOD COVERED October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Mucosal Immune Responses in Nonhuman primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Clinical Investigation

SECTION Mucosal Immunity Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To further define the basis of T cell function in the inductive and effector limbs of the normal intestinal immune system, the capacity of mucosal lymphocytes to produce and use lymphokines and their effects on regulation of immunoglobulin production were determined in normal nonhuman primates. Northern blots of RNA from mitogen-activated lamina propria T cells contained more mRNA for IL-2 and IFN-gamma than mesenteric lymph node T cells. There was much higher expression of IL-4 and IL-5 mRNA in both mesenteric lymph node and lamina propria T cells than in T cells from other sites. In studies of lymphokine utilization, T cells from lamina propria had high IL-2 induced but no IL-4 induced proliferative responses. In contrast, mesenteric lymph node T cells had high IL-4 induced and lower IL-2 induced proliferative responses compared to lamina propria T cells. The regulatory effects of these lymphokines were studied in pokeweed mitogen stimulated cultures; both IL-2 and IFN-gamma were shown to enhance immunoglobulin production, whereas IL-4 exhibited dose-dependent inhibition of immunoglobulin production. IFN-gamma reversed the inhibitory effect of IL-4. Lamina propria T cells had higher helper activity in pokeweed mitogen stimulated cultures and exhibited less inhibition by IL-4 than mesenteric lymph node T cells. These data and previous studies suggest that T cells in inductive sites such as the mesenteric lymph node are primarily "naive" cells which are less capable of producing lymphokines which cause terminal B cell differentiation, but this population is able to expand under the influence of multiple lymphokines such as IL-2 and IL-4. In contrast, the data suggest that T cells in the effector compartment of the lamina propria are comprised primarily of differentiated "memory" cells that have a limited ability to proliferate in resopnse to IL-4 but produce high levels of IL-2, IFN-gamma , IL-4 and IL-5 and have high helper activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00469-04 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complement: Studies in Viral Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Louis F. Fries, Medical Officer (Research), LCI/NIAID
Others: Michael Frank, Chief, LCI/NIAID
Robin McKenzie, Medical Staff Fellow, LCI/NIAID
Srikantan Vasantha, Visiting Fellow, LCI/NIAID
Brian Murphy, Senior Investigator, LID/NIAID
John Zurlo, Medical Staff Fellow, LCI/NIAID
Kathleen Coelingh, LID/NIAID

COOPERATING UNITS *(if any)*

Harvey M. Friedman, University of Pennsylvania; Gary H. Cohen, University of Pennsylvania; Roselyn J. Eisenberg, University of Pennsylvania

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 3.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In studies using bacterial systems we have found evidence suggesting that complement attack on a target particle is directed by the specificity and type of antibody that initiates the complement activation. This was originally suggested in studies using bacterial and red cell systems. Analysis of these systems proved to be highly complex and we decided to move to a more well defined system. The attachment of complement components to type 3 parainfluenza virus was chosen for study since this is a typical enveloped virus and has a well defined structure. Members of LID/NIAID were working with this virus and there were monoclonal antibodies available to many of its surface proteins.

Many viruses activate the complement cascade directly, this is generally not a neutralizing event in the absence of antibody. We used a nonneutralizing IgM monoclonal antibody to parainfluenza virus type 3 (PIV3) hemagglutininneuraminidase (HN) to explore the role of antibody in complement-dependent neutralization of PIV3. Neither the antibody nor nonimmune guinea pig serum (GPS) neutralized PIV3 significantly, but a more than 100-fold reduction in titer was found when antibody and GPS were combined. Heat-inactivated GPS or GPS lacking either of two different complement proteins were all inactive with or without antibody. Specific repletion of the deficient sera with highly purified complement proteins restored neutralizing activity, indicating an absolute requirement for the classical pathway of complement activation and lytic terminal complement components, and viral lysis was confirmed by electron microscopy. The presence of antibody before complement activation was essential; later addition had no effect. Spontaneous complement activation by PIV3 occurred via the classical pathway in the absence of antibody. Addition of antibody did not alter the overall rate or extent of complement component C3 binding to PIV3 in these experiments. We conclude that certain nonnneutralizing antibodies may support complement-dependent PIV3 neutralization by facilitating viral lysis. Lysis may require antibody-dependent localization of the membrane attack complex or reorganization of the viral envelope structures to facilitate attack complex insertion and lysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00470-04-LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chronic Epstein Barr VIrus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
OTHER:	J. Dale	Clinical Research Nurse	LCI,NIAID
	R. Holman	Medical Staff Fellow	LCI,NIAID

COOPERATING UNITS *(if any)*

M.Kruesi (LCS,NIMH), J.Grafman (LMN, NINCDS), M.Dalakas (NINCDS), M.Demitrack, P.Gold (NIMH)

LAB/BRANCH

Laboratory of Clinical Investigations

SECTION

Medical VIrology SEction

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 02892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus and to characterize multiple aspects of the chronic fatigue syndrome. To date this research project has involved nearly 150 patients. Included are 7 patients with severe chronic Epstein Barr virus infections. During the past year we used in situ hybridization to study EBV gene expression in the tissues of these severely ill patients. Tissues of some of the patients were proven to have increased levels of EBV RNA. On this basis and on the basis of other clinical, historical, and serologic features of these patients, we have proposed criteria by which severe chronic EBV infection can be diagnosed.

Detailed neurologic, neuroendocrinologic, and neuropsychologic studies are being conducted in selected patients with chronic fatigue. To date, we have found no consistent abnormalities in the chronic fatigue patients, although some can be shown to have immune abnormalities, depression, and other neuroses. Having shown acyclovir to not benefit patients with the chronic fatigue syndrome, we conducted a series of additional open therapeutic trials during the past year. Some patients showed marked improvements while on tricyclic or MAO inhibitor compounds. During the past year studies of the pituitary-adrenal responsiveness to corticotropin releasing hormone reveals statistically significant reductions in adrenal cortecol synthesis in patients with the chronic fatigue syndrome. Confirmatory studies using ACTH challenges are underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00481-04
LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptors and Transduction Mechanisms in Human Phagocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
Others:	John I. Gallin, M.D.	Scientific Director, DIR,	NIAID
	Daniel Rotrosen, M.D.	Senior Staff Fellow	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID
	Thomas L. Leto, Ph.D	Senior Staff Fellow	LCI/NIAID
	Hiroyki Nunoi, M.D., Ph.D.,	Visiting Associate	LCI/NIAID
	Michael Kleinberg, M.D.,Ph.D.,	Med. Staff Fellow	LCI/NIAID
	Karen J. Lomax, M.D.	Senior Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)* Elaine Gallin, Dept. Physiol., Arm. Forc. Rad. Biol. Inst.; John Albert, Mol. Vir. Immunol. Div., Georgetown Univ., Rockville, MD.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	4.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human neutrophils defend against infection and modulate inflammation. This project defines the biochemical structure and function of surface receptors, transduction proteins and effector systems involved in phagocyte responses to inflammation. Studies in neutrophils focus upon: (i) chemotactic receptors for formyl peptide (FPCR) and C5a; (ii) GTP binding regulatory proteins (Gi) required for receptor and effector functions; (iii) cytosolic proteins NCF-47K and NCF-65K and membrane cytochrome b_{558} (CYTO b) mediating superoxide production. We show that both FPCR and Gi are present and functionally coupled in an intracellular pool in neutrophils. Using myeloid cell mRNA in a frog oocyte expression system we demonstrate responses to FMLP and C5a which include chloride currents and calcium efflux. Size fractionation of mRNA shows that transcripts encoding FMLP and C5a receptor are 2 kb. We have delineated, purified, and cloned cDNA encoding two neutrophil cytosol factors of 47 kD and 65 kD (NCF-47K and NCF-65K) which are essential effector elements required for activation of the neutrophil respiratory burst. NCF-47K is predicted to be a 41.9 kD basic protein with an arginine and serine rich carboxyterminus containing phosphorylation sites and shares a small region of homology with ras p21 GTPase activating protein (GAP). NCF-65K is predicted to be a 60.5 kD acidic protein. NCF-47K phosphorylation is regulated by GTP and by a cytoplasmic C-terminal functional domain of CYTO b we have defined. In other studies we show that glycosylation of CYTO b differs in neutrophils and eosinophils. These studies enhance understanding of neutrophils in inflammation and may lead to development of antiinflammatory agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00495-03 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Robert J. Hohman, Ph.D.	Expert	LCI/NIAID
	Juan L. Rodriquez, M.D.	NRSA Fellow	LCI/NIAID
	Raynaldo A. Martin	Biologist	LCI/NIAID
	Thomas Hultsch	Special Volunteer	LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.85	1.1	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

IgE receptor-mediated phosphatidylinositol hydrolysis was studied in rat basophilic leukemia cells permeabilized with alpha toxin from S. aureus. The rate of PI hydrolysis in permeabilized cells is twice that observed in intact cells. As we previously demonstrated for IgE receptor-mediated degranulation of RBL cells permeabilized with alpha toxin, receptor-mediated phosphatidylinositol hydrolysis in permeabilized cells proceeds if Ca^{2+} is omitted from the buffer. In addition, permeabilized cells are extremely sensitive to small concentrations of selected anions. For example, 1 mM glutamate causes a 2 fold enhancement of receptor-mediated phosphatidylinositol hydrolysis, while 2 mM sulfate inhibits the reaction by >60%. Moreover, the relatively high concentrations of chloride often used by investigators when studying permeabilized cells are also inhibitory.

Diisopropylfluorophosphate (DFP), an irreversible inhibitor of serine esterases, inhibits IgE receptor-mediated phosphatidylinositol hydrolysis in a dose-dependent manner with 50% inhibition occurring at 3 mM DFP. DFP must be present when the cells are triggered for it to have an inhibitory effect. Therefore, if cells are incubated with DFP, washed, and triggered in the absence of DFP, there is no inhibition. In addition to inhibition of receptor-mediated phosphatidylinositol hydrolysis in intact cells, incubation of RBL cells with DFP results in inhibition of phospholipase C activity when the treated cells are used to make a cytosolic extract and the phospholipase C activity is measured. Moreover, studies performed on purified phospholipase C demonstrate that this enzyme is directly inhibited by the presence of DFP in the assay. These results suggest that DFP is able to bind to and inhibit phospholipase C. This finding could is consistent with all of the data concerning inhibition of mast cell activation by DFP and could explain the effect of this inhibitor on IgE receptor-mediated exocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00496-03 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between the Human Immunodeficiency Virus (HIV) and Herpesviruses.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.M. Ostrove	Senior Staff Fellow, LCI, NIAID
	S. Nagpal	Visiting Fellow, LCI, NIAID
	D. Margolis	Medical Staff Fellow, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of hrepesviruses as a possible cofactor in the progression and oathogenesis of the acquired immune deficiency syndrome (AIDS) are being investigated. AIDS patients develop a wide variety of opportunistic infections and we have been studying the interactions of herpes simplex (HSV) and varicella-zoster (VZV) viruses with the human immune deficiency virus (HIV) in controlled laboratory experiments. We have demonstrated that plasmids containing HIV-LTR-CAT were significantly stimulated by herpes simplex virus types 1 and 2 (HSV) and varicella zoster virus (VZV) infections. Two HSV genes, ICP4 and ICP0 and two VZV genes, genes 4 and 62, were shown to be responsible for this effect. The stimulation seen with these HSV and VZV genes differed depending on which cell type was used implying that cellular proteins were playing a major role in the HIV activation seen by herpesviruses. Analysis of mutants within the HIV-LTR have helped map the regions required for HSV activation to the SP1 and NF-kB binding regions. HSV infection was shown to stimulate the release of active NF-kB which is required for HIV LTR directed transcription. We have recently identified two VZV genes that have the ability to inhibit the activation of HIV-LTR-CAT by VZV genes 4 and 62. This inhibition is specific in that these genes do not inhibit the activation of HTLV-1 by the pX gene product. Inhibition appears to be at the transcriptional level as demonstrated by the lack of increase in steady-state HIV LTR CAT RNA levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00513-02 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Arnold S. Kirshenbaum, M.D.	NRSA	LCI/NIAID
	Helen Thompson, Ph.D.	Visiting Fellow	LCI/NIAID
	John Costa, M.D.	Medical Stff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*

NIDR (P. Burbelo); NIAMS (J.P. Kinet)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.50	3.00	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dexamethasone inhibits DNA, RNA and protein synthesis in the mouse PT18 mast cell line. Inhibition of thymidine incorporation was limited to the glucocorticoid class of steroids. Human bone marrow grown on agar in the presence of IL-3 gives rise to macrophages that ingest agar and subsequently may be falsely identified as mast cells due to the metachromatic properties of the ingested agar matrix. Co-culture of rat connective tissue mast cells and rat fibroblasts generates macrophage-colony stimulating factor. Cultured mouse mast cells and human basophils adhere to laminin (with P. Burbelo). Adherence requires activation with PMA, ionophore, or via IgE-dependent mechanisms. Histamine release is not required. In mast cells, Northern analysis showed a high level of mRNA for a 32 kD laminin receptor. The synthetic laminin A chain derived peptide PA22-2 substituted for laminin. Human basophil adherence was inhibited by anti-laminin antibody and by CD18 antibody which recognizes the common β-subunit of the LeuCam family of adhesion molecules. Mouse mast cells chemotax to laminin and the peptide PA22-2. Chemotaxis is enhanced by cell activation but independent of degranulation. The gamma subunit of the high affinity IgE receptor was cloned and sequenced from a human basophil DNA library. Message for the high affinity IgE receptor subunits increases with time in both mouse and human bone marrow culture, suggesting that the appearance of IgE receptors precedes the expression of the mast cell or basophil phenotype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00514-02 LCI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis and Treatment of Adverse Reactions to Foods and Additives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID

COOPERATING UNITS *(if any)*

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

On a collaborative project, with Dr. P.V. Subba Rao at the Indian Institute of Science, we have previously isolated and characterized three antigens from shrimp.

We have now examined idiotypic, anti-idiotypic, and anti-anti-idiotypic responses to one shrimp allergen, SA-II. Balb/c mice were immunized with idiotypic antibodies directed against SA-II resulting in anti-idiotypic antibodies. These anti-idiotypic antibodies recognized shrimp-specific human idiotypic antibodies of the IgE isotype from 18 of 20, and IgG antibodies from 14 of 20 shrimp-sensitive patients. SA-II specific anti-idiotypic antibodies induced anti-SA-II IgE and IgG responses in the absence of allergen. Thus, anti-idiotypic antibodies raised against SA-II-specific idiotypic antibodies substitute for SA-II in the induction of SA-II specific idiotypic antibodies. The demonstration of shared idiotopes on IgG and IgE antibodies in the sera of shrimp-sensitive patients supports the use of allergen-specific anti-idiotypic antibodies as surrogate allergens.

We have completed a clinical study to determine the reproducibility of "allergic" reactions to aspartame, a non-nutritive sweetener. Although efforts to notify potential subjects of this protocol by means of notices in newspapers and information presented to local physicians continued over 2 and 1/2 years, we received only approximately 50 inquiries. Twelve of these individuals underwent blinded challenge. Only two had mild reactions during challenge, possibly due to aspartame. Aspartame allergy seems to be a rare clinical problem.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00521-02 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John I. Gallin, M.D.	Director, DIR,	NIAID
Others:	Joan M.G. Sechler		LCI/NIAID
	Harry L. Malech, M.D.	Head, Bacterial Dis. Sec.	LCI/NIAID
	Stuart Abramson, M.D, Ph.D.	Medical Staff Fellow	LCI/NIAID
	Karen J. Lomax, M.D.	Senior Staff Fellow	LCI/NIAID
	Phil Murphy, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS (if any) D Melnick, DIR/NIAID; T Nutman and C King, LPD/NIAID; J Rex and J Bennett, LCI/NIAID

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study effects of cytokines upon host defense and upon phagocytic cell function. Interferon gamma (IFN-g) corrected abnormal oxidative metabolism and bactericidal and fungicidal activity of neutrophils and monocytes from patients with chronic granulomatous diseases of childhood (CGD) whether administered in vivo or in vitro. Twenty five CGD patients at NIH have been entered into a double blinded study of the efficacy of prophylactic IFN-g in preventing infection. IFN-g treated normal monocytes increased mRNA transcripts and protein levels of membrane and cytoplasmic components of the NADPH oxidase system. B-cells from patients with Hyperimmunoglobulin E and Recurrent Infections (Job's) Syndrome (HIE) have abnormally high spontaneous in vitro synthesis of IgE. In vivo administration of IFN-g to HIE patients results in a marked decrease in spontaneous in vitro production of IgE by B-cells and in some patients resulted in a decrease in serum IgE. Production of IgG1,3, and 4, but not IgG2 or IgM was also decreased by IFN-g. Interleukin 4 (IL-4) treatment of macrophages in culture inhibited the ability of activated cells to produce superoxide, and blunts, but does not eliminate IFN-g enhancement of respiratory burst responses. IFN-g induces monoycte polykaryon formation, in an in vitro model of granuloma formation, through a cascade of events involving interleukin-1 and tumor necrosis factor, an effect inhibitable by corticosteroids. We showed that human macrophage cell lines can be established without transformation and that CSF-1 produced by these cultured cells likely facilitates human monocyte replication in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00548-01 LCI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prevention of Genital Herpes Simplex Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator	LCI,NIAID
OTHER:	A. Freifeld	Guest Researcher	LCI,NIAID
	J.Dale	Clinical Research Nurse	LCI,NIAID
	M.Murray	Clinical Research Nurse	LCI,NIAID

COOPERATING UNITS *(if any)*
R.L.Burke,C.Dekker (Biocene, Inc., Emeryville, CA)
L.Corey (University of Washington, Seattle), A Nahmias (Emory University, Atlanta GA)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Having completed a decade of work on antiviral treatment of genital herpes, we turned this year to studies of disease prevention. Two protocols were finalized. The first is a multi-center study of acyclovir for prophylaxis of genital herpes in high-risk, suceptible adults. The second study, which will formally begin during the next research period, is a phase I assessment of a recombinant HSV-2 glycoprotein vaccine. In the coming year-and-a-half, the safety and immunogenicity of the vaccine will be explored in patients with and without prior herpes infection.

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LABORATORY OF IMMUNOGENETICS
1989 Annual Report
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ANNUAL REPORT

LABORATORY OF IMMUNOGENETICS
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1988 to September 30, 1989

RESEARCH PROGRESS

Programs in the Laboratory of Immunogenetics continue to emphasize the gene families that are important in the control of immune function. In addition studies concerning the role of retroviruses that exercise deleterious effects on the immune system are in progress. These studies utilize basic methodology in molecular biology, genetics and immunology.

Normal and Pathologic Immune Responses in the Laboratory Rabbit

Studies continue on the immune system of the rabbit. These include an investigation of the major histocompatibility complex (MHC) of this species and studies on the lymphoid cell populations and the markers that can be used to identify them. Recent investigations concern the infection of rabbits with the human retroviruses HIV-1 and HTLV-I and the effects of these pathogens on immune responsiveness. These investigations have led to development of a model for the study of acquired immunodeficiency syndrome (AIDS) and provide a basic tool to gain information about the immune system of the rabbit.

Studies of Rabbit MHC Genes

Recent studies on the major histocompatibility complex of the rabbit (RLA) have focused upon development of reproducible methods to type rabbits for the MHC. It is desired to use rabbits in immunologic experiments that require matching at both class I and class II loci. The use of molecular typing procedures for both class I and II genes has allowed success in this area. An extensive family study was carried out using two class I probes and four class II α chain probes (**Marche, Rebiere**). Molecular genetic typing has defined 13 distinct haplotypes which resulted from a combination of 11 RLA-A (class I) and 10 RLA-D (class II) regions. Among class II genes, DQα showed the highest level of polymorphism, 9 of 10 D regions could be distinguished by DQ hybridization. Several haplotypes were found identical with respect to either class I or class II genes suggesting that class I genes are clustered in the A subregion and class II genes in the D subregion and that these haplotypes have resulted from recombinations between the A and D subregions. Unlike the murine MHC where class I gene clusters are localized on both sides of the H-2I subregion, the rabbit, like man, appears to have discrete class I and class II regions. Results of random typing of colonies at the NIH and the Institut Pasteur suggest that there are only a limited number of molecularly defined haplotypes in addition to those defined here.

The complete sequence of a genomic clone for RLA-DRα was determined (**Marche, C. LeGuern**). Alignment of the deduced amino acid sequence with other class II α chains indicated that the two cysteine residues (positions 107 and 163) and two putative sites of N-glycosylation (Asn 78 and Asn 118) are conserved. The RLA-DRα promoter region was localized by alignment with the 5' untranslated region of the HLA-DRα gene. A potential CAP site was assigned 63 bp upstream of the initiation codon. Twenty nine bp upstream of this position a TCTAT stretch was found that may correspond to an atypical TATA box. Comparisons of 5' untranslated regions have allowed definitions of conserved class II sequences namely the octamer and the X and Y boxes. Conservation of these sequences in the rabbit DR upstream sequence reinforces their putative role in the regulation of transcription of class II genes.

Current studies focus on the use of MHC matched rabbits for cellular studies involving cytolytic T-cell activity to various proteins including the nef and env products of the HIV-1 virus (**A. LeGuern**).

Lymphoid Cell Populations in the Rabbit

Studies aimed at providing structural and functional definition of lymphoid cell populations in the rabbit have continued. Approaches taken have included characterization of available antibodies, attempts to clone rabbit homologs of known markers and transfection of rabbit cell lines with genes for T-cell markers of man. A new monoclonal antibody to rabbit IgM was obtained from collaborators and shown to react specifically with B cells. This will replace the polyclonal sera that are used for the purpose of identifying cells with surface Ig. An antibody that reacts with activated T-cells was obtained by immunization with cells from the appendix (**Gordon, Roy**). This marker is expressed on certain cell lines and is represented at varying degrees in PCMB, spleen and lymph nodes but not in thymus. This suggests that it may react with the IL-2 receptor and studies are underway to prove this.

A cDNA library has been screened for clones that cross-hybridize with human CD4 genes. Several candidates have been picked and these are being studied in an effort to characterize the rabbit CD4 homolog (**Lee**). Transfection studies are being carried out to obtain a rabbit cell line that expresses the human CD4 gene (**Hague**). It will be learned whether this transfectant is a significantly better host for HIV-1 than the native line or the line transfected with irrelevant genes. If the presence of CD4 is a major determinant in the ability of rabbit cells to be infected with HIV-1, transgenic rabbits will be prepared with CD4 as transgene.

Action of HTLV-I in vitro and in vivo

Transformed cells lines have been obtained from rabbits infected with HTLV-I. Lines were derived from animals three and 36 weeks after injection of the rabbits with irradiated MT-2 cells and one was IL-2 independent whereas the other retained its dependency (**Kulaga, Lubin**). Both lines were shown to be clonal by Southern blot analysis of the viral genome integrated into the cell DNA and both expressed rabbit T-cell markers. PBMC and spleens from HTLV-I infected rabbits were shown to be infected with HTLV-1 by polymerase chain reaction amplification of cellular DNA using primers for gag, env and tax genes. One rabbit doubly infected with HIV-1 and HTLV-I was shown to have both viruses in spleen and PBMC DNA samples taken nearly one year after infection (**Truckenmiller**). More complete characterization of these cells will be carried out along with comparisons to normal lymphoid cells.

A rabbit macrophage line 6083 was shown to have susceptibility to infection with HIV-1 by a number of criteria but the supernatant was consistently negative for reverse transcriptase which is a hallmark of HIV-1 infection. Examination of supernatants from noninfected 6083 cells revealed the presence of a substance that inhibited RT activity in control samples. Partial characterization of this inhibitor shows that it is non-dialyzable, heat labile and that it acts not on the enzyme itself, but rather on the substrates used by RT (**Recker**).

Infection of Rabbits with HIV-1

Injection of rabbits with a human T-cell line that has been infected with HIV-1 causes seroconversion within 4 weeks of injection (**Kulaga**). Normally 5 x 10^6 cells are injected, if higher doses are used the seroconversion occurs earlier and levels of antibody are elevated. Forty seven rabbits have been successfully infected. One animal did not seroconvert after the original dose of infected cells but antibodies could be detected following a booster injection. Virus has been detected by polymerase chain reaction amplification of viral DNA in rabbit PBMC and spleen. Positive reactions were seen after culture in IL-2 for several weeks and in a number of cases could be found in cells or tissue directly from the animal. Immune response to tetanus toxoid and to BCG have been monitored in infected animals and were shown to be diminished or absent in rabbits that were infected with HIV-1 following immunization (**Gordon**). Responses were measured by determination of antibody levels and by intradermal challenge with PPD. In other experiments the effects of injection with infected human blood samples is being tested. Initial results indicate that antibodies raised by such injections may crossreact with human cell products that are present on plates supplied with the commercial ELISA kit used in the study (**Recker**). Test are in progress to determine whether recombinant HIV-1 proteins can be used to prepare plates to circumvent this problem so that the rabbit may be used for diagnostic tests to determine infection with HIV-1.

T-cell Receptor Genes in Man

The laboratory has pioneered in the studies of genetic markers for the T cell receptor (Tcr) gene complex in man (**Robinson**). In the past year, significant progress has been made in these studies. Linkage has been established between genes in the Tcr β complex and the remitting relapsing form of Multiple Sclerosis (**Hauser**). The basic studies of polymorphism have been advanced by discovery of variants that involve insertion or deletion of 15 to 30 kilobase stretches of DNA within this complex. In addition variants involving single base interchanges in V region gene segments have been mapped in families and populations.

Disease Association of Tcr Genes in Man

In order to investigate the genetic control of human immune responses, inheritance patterns of several genes known to play important roles in a variety of immune processes have been analyzed in human families. HLA and Tcr genes have been examined by Southern blot analyses using specific DNA probes to identify restriction fragment length polymorphism (RFLP). In addition, direct sequence comparisons of Tcr variable (V) region genes was achieved by amplification of genomic DNA using the polymerase chain reaction (PCR) procedure, cloning into m13 and performing DNA sequence analysis. Once sequence differences were characterized, allele specific oligonucleotides were designed to hybridize with the region including substitutions and were used as probes to test PCR amplified DNA samples. Examination of the Vβ1 gene revealed only a single nucleotide substitution that resulted in an amino acid substitution located within a hypervariable region. Correlation between RFLP and this sequence difference was not precise.

In contrast to the limited polymorphism observed with individual markers, there is considerable polymorphism in the combination of markers inherited together in both Tcrα (Grier) and Tcrβ haplotypes. These observations have an important impact upon studies to determine whether Tcr genes are associated with disease susceptibility and indicate that studies of families showing a predisposition to a particular disease will be most informative. The inheritance of Tcrβ haplotypes was analyzed in families of 40 sibling pairs concordant for the relapsing/remitting form of Multiple Sclerosis (MS). The mean proportion to Tcrβ haplotypes identical by descent inherited by MS sibling pairs was significantly increased compared with expected values (means test $p<0.004$) and one allelic form of a Tcrβ V region gene segment was overrepresented on MS chromosomes. These results demonstrate that a gene within the TCRb complex or a closely linked locus influences susceptibility to MS.

Additional studies in this area have used the technique of pulse field gel electrophoresis to study genes in the Tcrβ region. Two distinct polymorphisms were found by this method and these were shown to result from two independent deletion or insertion events that have occurred in the evolutionary history of this complex. The gene frequencies of the polymorphic forms were approximately equal suggesting that the events have not occurred in recent times.

Molecular and Functional Analysis of Class II Antigens

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded in the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. How the processed peptides are generated and where they interact with MHC molecules is largely unknown. Attempts are being made to define the function of human class II antigens in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens. The interaction between a class II MHC molecule and a specific T cells is complex because it involves (i) an immunogenic peptide, (ii) an antigen-specific T-cell receptor (TCR) and (iii) the CD4 molecule expressed on the T cell. In order to dissect these interactions an assay has been developed that measures a functional CD4-class II interaction in the absence of a TCR-class II interaction. Using this assay, it has been demonstrated that both HLA-DR and -DP antigens can interact with CD4 and that the site on CD4 involved in this interaction is distinct from that to which HIV binds (**Karp, Sekaly**).

Class II Molecules and Antigen Presentation

An experimental model has been developed to test the basis of the distinction between class I and class II MHC-restricted antigen presentation. It has been proposed that the invariant chain associated intracellularly with class II MHC molecules may control this distinction by preventing the binding of endogenously derived peptides to class II molecules. Transfected cells expressing class II molecules in absence or in presence of the invariant chain have been generated (**Long**). T cell clones and lines specific for influenza virus antigens have been generated that are restricted by the HLA-DR1 molecule expressed on the transfected cells (**Jaraquemada**). This material can be used to test directly whether the invariant chain plays a role in tolerance to self by preventing the presentation of endogenous antigens by class II molecules (**Jendoubi**).

Gene Expression In LAK Cells

Peripheral blood lymphocytes grown in the presence of high doses of interleukin-2 (IL-2) acquire the ability to lyse tumor cells. This cytolytic activity is not restricted by antigens encoded in the major histocompatibility complex (MHC) and is observed with a variety of both autologous and allogeneic tumors. This activity has been called lymphokine-activated killing (LAK) and has been used in clinical treatment of tumors. The origin of cells with LAK activity and the mechanism by which they recognize and kill their targets are unknown. Most of the cells with LAK activity display the $CD3^{-}CD4^{-}CD8^{-}CD16^{+}$ phenotype. Populations of cells with this phenotype were purified by a combination of antibody mediated complement killing and cell sorting. Clones were also obtained by limiting dilution. RNA hybridizations demonstrated that these cells express only the CD3ε chain of the CD3 complex and the δ gene of the Tcr complex. The Tcr δ transcripts were derived from unrearranged genes. These observations are consistent with the notion that these MHC-unrestricted cytotoxic cells are derived from an early stage in the T cell lineage. An antibody has been isolated that binds to a cell surface molecule on these cells, and that can modulate their cytotoxic activity. A cloning strategy based on cDNA expression is in use to isolate the gene encoding this cell surface molecule (**Biassoni, Malnati**).

Gene Regulation Studies

Investigations in this area currently focus on the regulation of the J chain gene in B lymphocytes and the function of the J chain protein. The human J chain gene was cloned several years ago to compare its regulation to that of immunoglobulin genes (**Max**). Analysis of the promoter has in the past year clarified three regions essential for promoter function; deletion of any one of these regions reduces by about 90% the expression of a reporter gene transfected into the murine myeloma S194. One region, about 400 bp upstream of the transcriptional initiation site, contains three repeats of the hexamer CCTTCA. The second, about 300 bp upstream of the initiation site, contains a "κB" element similar to those found in regulatory sequences of the immunoglobulin κ gene, the human immunodeficiency virus, and several other genes of the immune system. The third region contains a hexamer identical to a site in the murine J chain promoter that has been shown to bind a B cell-specific nuclear protein. The function of the J chain is being explored by determining the immunologic consequences of the loss of J chain gene function. Because no natural J chain deficiencies have been described, a mouse strain will be created in which the normal J chain gene has been inactivated. In initial experiments a modified mouse J chain gene has been transfected into mouse embryonic stem cells. Cells in which the inactive J chain gene construct has replaced a copy of the normal gene by homologous recombination will be used to derive live mice with no active J chain gene. The immunologic consequences of this loss will be examined.

LABORATORY OF IMMUNOGENETICS
Annual Report
October 1, 1988 to September 30, 1989

ADMINISTRATIVE REPORT

In the past year Dr. Eric Long has been granted tenure and has officially assumed the leadership of the Molecular Immunology section within the laboratory. Dr. Edward Max has accepted a position at the FDA and will leave LIG in the summer of 89. Dr. Bishop Hague has joined the Immunogenetics Research section of the lab after taking his Ph.D. from the University of Massachusetts, Dr. Annie LeGuern joined this section with a fellowship from the Institut Pasteur. Dr. Marie Christine Rebiere left the laboratory during this time. Dr. Mauro Malnati from the National Institute for Cancer Research in Genoa, Italy, and Dr. Moncef Jendoubi from the Institut Pasteur in Paris, France, have joined the Molecular Immunology section of LIG. Dr. Roberto Biassoni returned to Italy. Dr. Rafick Sekaly left to assume a position in the Clinical Research Institute of Montreal, Canada.

LABORATORY OF IMMUNOGENETICS

Annual Report
October 1, 1988 to September 30, 1989

HONORS AND AWARDS

In the past year Dr. Kindt has presented invited talks concerning laboratory studies at the meeting of the Laboratory of Tumor Cell Biology, at the AIDS National Cooperative Drug Discovery and Development meeting in Oakland CA, at the La Jolla meeting on Animal Models for AIDS and at the 7th International Congress of Immunology. Data were presented in poster format at the FASEB meeting in New Orleans, and at the International AIDS Conference in Montreal. Dr. Kindt presented the Dorothy Baugh Harmon lecture at the Oklahoma Medical Research Foundation and seminars were given at the University of Illinois at Chicago, the University of Texas at Dallas, The Rockefeller University, Genentech Inc., Viagene Inc., Hybritech Inc., and IDEC Pharmaceuticals Inc. He continues to serve on the review board for the Multiple Sclerosis Society, served on the Israel Cancer Research Fund, and has been appointed to the Board of Scientific Visitors for the Oklahoma Medical Research Foundation. Dr. Kindt serves as a scientific advisor to Oncor Inc. and to Southern Biotechnology Inc., and has been appointed Head of the Scientific Advisory Board of RRI Inc. Dr. Kindt serves as deputy editor of the Journal of Immunology, and is on the board of the Journal of Experimental Medicine, Current Opinion in Immunology and is regional editor for Research in Immunology (formerly Annals of Immunology).

Dr. Eric Long presented lectures at the University of Lausanne, Switzerland, at the University of Geneva, Switzerland, at the Immunology Branch of the National Cancer Institute, at the 3rd Biotechnology Symposium of the University of Montreal, Canada, and at the Symposium on Molecular Basis of Allorecognition, in Milwaukee. He chaired a workshop at the 7th International Congress of Immunology in Berlin and presented an invited talk in a separate workshop. Data were presented in poster form at the UCLA-Symposium in Steamboat Springs, the 15th meeting of the American Society for Histocompatibility and Immunogenetics in Toronto and at the FASEB meeting in New Orleans. He serves as Associate Editor for the Journal of Immunology, and Section Editor for Current Opinion in Immunology. He has served as a Guest Editor for a special symposium in Immunologic Research. Dr. Long serves on the NIAID Personnel Advisory Committee and the NIAID Transgenic Mouse Oversight Committee.

Dr. Mary Ann Robinson presented invited lectures at Frederick Cancer Research Center and at the Walter Reed Medical Center and presented current laboratory data at the ASHI meeting in October. Posters were presented at the ASHI and FASEB meetings. Dr. Robinson served on the Abstract Review and Selection Committee for the ASHI meeting and is presently a member of the Awards Committee. She also served as an Adhoc Reviewer for the United States-Israel Binational Science Foundation. Dr. Robinson serves on the NIAID Safety Committee and on the Introduction to Biomedical Research Selection committee.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00166-12-LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG/NIAID
Others:	Ira Lubin	IRTA	LIG/NIAID
	Annie LeGuern	Guest Worker	LIG/NIAID

COOPERATING UNITS (if any)

Patrice Marche, Institut Pasteur, Paris; Christian LeGuern, Immunol. Branch, NCI

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent studies on the major histocompatibility complex (RLA) of the rabbit have focused upon development of reproducible methods to type rabbits for the MHC. It is desired to use rabbits in immunologic experiments that require matching at both class I and class II loci. The use of molecular typing procedures for both class I and II genes have allowed some success in this area. An extensive family study was carried out using two class I probes and four class II α chain probes. It was found that the use of the two class I and two of the class II probes with three restriction enzyme digest will allow typing for most haplotypes encountered in our colony and those of collaborators at the Institut Pasteur in Paris. Sequence determination of a RLA-DRα gene was completed and compared to similar class II genes of man and mouse. It was found that the RLA gene was most similar to the human DRα. In addition, it was possible to locate the enhancers and other signal sequences in this gene and to show their high level of interspecies conservation. Current studies focus on the use of MHC matched rabbits for cellular studies involving cytolytic T-cell activity to various proteins including the nef and env products of the HIV-1 virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00168-12-LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG/NIAID
Others:	Mark Gordon	NRC Fellow	LIG/NIAID
	Kevin Lee	NRC Fellow	LIG/NIAID
	Bishop Hague	IRTA	LIG/NIAID

COOPERATING UNITS (if any)

Mike Roy, Walter Reed Army Hospital; Richard Goldsby, University of Massachusetts, Amherst. Chamer Wei, Transgenic Sciences Inc., Worcester, MA.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies aimed at providing a structural and functional definition of lymphoid cell populations in the rabbit have continued. Approaches taken have included characterization of available antibodies, attempts to clone rabbit homologs of known markers and transfection of rabbit cell lines with genes for T-cell markers of man. A new monoclonal antibody to rabbit IgM was obtained from collaborators and shown to react specifically with B cells. This will replace the polyclonal sera that are used for the purpose of identifying cells with surface Ig. An antibody that reacts with activated T-cells was obtained by immunization with cells from the appendix. This marker is expressed on certain cell lines and is represented at varying degrees in PCMB, spleen and lymph nodes but not in thymus. This suggests that it may react with the IL-2 receptor and studies are underway to prove this. A cDNA library has been screened for clones that cross hybridize with human CD4 genes. Several candidates have been picked and these are being studied in an effort to characterize the rabbit CD4 homolog. Transfection studies are being carried out to obtain a rabbit cell line that expresses the human CD4 gene. It will be learned whether this transfectant is a significantly better host for HIV-1 than the native line or the line transfected with irrelevant genes. If the presence of CD4 is a major determinant in the ability of rabbit cells to be infected with HIV-1 transgenic rabbits will be prepared with CD4 as transgene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-A1-00170-12 LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eric O. Long	Visiting Scientist	LIG/NIAID
Others:	David Karp	Medical Staff Fellow	LIG/NIAID
	Dolores Jaraquemada	Visiting Fellow	LIG/NIAID
	Moncef Jendoubi	Visiting Fellow	LIG/NIAID

COOPERATING UNITS (if any)

Neuroimmunology Branch, NINCDS, NIH, W. Biddison, H. McFarland; Clinical Research Institute of Montreal, Montreal, Canada, R. Sekaly.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded in the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. How the processed peptides are generated and where they interact with MHC molecules is largely unknown. The aim of this project is to define the function of human class II antigens in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens.

The interaction between a class II MHC molecule and a specific T cell is complex because it involves (i) an immunogenic peptide, (ii) an antigen-specific T-cell receptor (TCR) and (iii) the CD4 molecule expressed on the T cell. In order to dissect these interactions an assay has been developed that measures a functional CD4-class II interaction in the absence of a TCR-class II interaction. Using this assay, it has been demonstrated that both HLA-DR and -DP antigens can interact with CD4 and that the site on CD4 involved in this interaction is distinct from that to which HIV binds.

An experimental model has been developed to test the basis of the distinction between class I and class II MHC-restricted antigen presentation. It has been proposed that the invariant chain associated intracellularly with class II MHC molecules may control this distinction by preventing the binding of endogenously derived peptides to class II molecules. Transfected cells expressing class II molecules in absence or in presence of the invariant chain have been generated. T cell clones and lines specific for influenza virus antigens have been generated that are restricted by the HLA-DR1 molecule expressed on the transfected cells. This material can be used to test directly whether the invariant chain plays a role in tolerance to self by preventing the presentation of endogenous antigens by class II molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00171-12 LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infection of Rabbits with Human Immunodeficiency Virus 1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG/NIAID
Others:	David Recker	Medical Staff Fellow	LIG/NIAID
	Mark Gordon	NRC Fellow	LIG/NIAID
	Annie LeGuern	Guest Worker	LIG/NIAID
	M.E. Truckenmiller	IRTA	LIG/NIAID

COOPERATING UNITS (if any)

Thomas Folks, CDC, Atlanta; Alan Lock, Div. Vet. Path., NIH; Henrietta Kulaga, Neuropsychiatry Br., NIMH.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	2.5	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Injection of rabbits with a human T cell line that has been infected with HIV 1 causes seroconversion within 4 weeks of injection. Normally 5 x 10^6 cells are injected, if higher doses are used the seroconversion occurs earlier and levels of antibody are elevated. Forty seven rabbits have been successfully infected. One animal did not seroconvert after the original dose of infected cells but antibodies could be detected following a booster injection. Virus has been detected by polymerase chain reaction amplification of viral DNA in rabbit PBMC and spleen. Positive reactions were seen after culture in IL-2 for several weeks and in a number of cases could be found in cells or tissue directly from the animal. Immune responses to tetanus toxoid and to BCG have been monitored in infected animals and were shown to be diminished or absent in rabbits that were infected with HIV 1 following immunization. Responses were measured by determination of antibody levels and by intradermal challenge with PPD. In other experiments the effects of injection with infected human blood samples is being tested. Initial results indicate that antibodies raised by such injections may crossreact with human cell products that are present on plates supplied with the commercial ELISA kit used in the study. Tests are in progress to determine whether recombinant HIV 1 proteins can be used to prepare plates to circumvent this problem so that the rabbit may be used for diagnostic tests to determine infection with HIV 1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01A1-00173-12-LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward E. Max Commissioned Officer LIG, NIAID

COOPERATING UNITS (if any)

Dr. John Seidman and Dr. Phil McCarthy, Department of Genetics, Harvard Medical School; Dr. James Flanagan, LDMI, NICHD; Dr. William Paul, LI, NIAID

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations in this group currently focus on the regulation of the J chain gene in B lymphocytes and the function of the J chain protein. The human J chain gene was cloned in our lab several years ago to compare its regulation to that of immunoglobulin genes. Our analysis of the promoter has in the past year clarified three regions essential for promoter function; deletion of any one of these regions reduces by about 90% the expression of a reporter gene transfected into the murine myeloma S194. One region, about 400 bp upstream of the transcriptional initiation site, contains three repeats of the hexamer CCTTCA. The second, about 300 bp upstream of the initiation site, contains a "κB" element similar to those found in regulatory sequences of the immunoglobulin κ gene, the Human Immunodeficiency Virus, and several other genes of the immune system. The third region contains a hexamer identical to a site in the murine J chain promoter that has been shown to bind a B cell-specific nuclear protein. We are also attempting to explore the function of the J chain by determining the immunologic consequences of the loss of J chain gene function. Because no natural J chain deficiencies have been described, we are attempting to create a mouse strain in which the normal J chain gene has been inactivated. To this end we have constructed a modified mouse J chain gene which has been transfected into mouse embryonic stem cells. Cells in which our inactive J chain gene construct has replaced a copy of the normal gene by homologous recombination will be used to derive live mice with no active J chain gene. The immunologic consequences of this loss will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-A1-00180-11-LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI:	Thomas J. Kindt	Chief	LIG/NIAID
Others:	M.E. Truckenmiller	IRTA	LIG/NIAID
	Bishop Hague	IRTA	LIG/NIAID
	David Recker	Medical Staff Fellow	LIG/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.4	PROFESSIONAL: 1.4	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transformed cells lines have been obtained from rabbits infected with HTLV-I. Lines were derived from animals three and 36 weeks after injection of the rabbits with irradiated MT-2 cells and one was IL-2 independent whereas the other retained its dependency. Both lines were shown to be clonal by Southern blot analysis of the viral genome integrated into the cell DNA and both expressed rabbit T-cell markers. PBMC and spleens from HTLV-I infected rabbits were shown to be infected with HTLV-1 by polymerase chain reaction amplification of cellular DNA using primers for gag, env and tax genes. One rabbit doubly infected with HIV-1 and HTLV-I was shown to have both viruses in spleen and PBMC DNA samples taken nearly one year after infection. More complete characterization of these cells will be carried out along with comparisons to normal lymphoid cells. A rabbit macrophage line 6083 was shown to have susceptibility to infection with HIV-1 by a number of criteria but the supernatant was consistently negative for reverse transcriptase which is a hallmark of HIV-1 infection. Examination of supernatants from noninfected 6083 cells revealed the presence of a substance that inhibited RT activity in control samples. Partial characterization of this inhibitor shows that it is non dialyzable, heat labile and that it acts not on the enzyme itself, but rather on the substrates used by RT.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00389-06-LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genotype Analyses of HLA and TCR Genes in Human Families

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mary Ann Robinson	Expert	LIG/NIAID
Others:	Alice Grier	IRTA	LIG/NIAID

COOPERATING UNITS *(if any)*

D. Bernard Amos, Duke University; S. Hauser, Massachusetts General Hospital; D. Posnett, Cornell University Medical Center

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to investigate the genetic control of human immune responses, inheritance patterns of several genes known to play important roles in a variety of immune processes have been analyzed in human families. HLA and T cell receptor (TCRα and TCRβ) genes have been examined by Southern blot analyses using specific DNA probes to identify restriction fragment length polymorphism (RFLP). In addition, direct sequence comparisons of TCR variable (V) region genes were performed by amplification of genomic DNA using the polymerase chain reaction (PCR) procedure, cloning into m13, DNA sequence analysis. Once sequence differences had been characterized, allele specific oligonucleotides were designed to hybridize with the region including the substitution and were used as proves to test PCR amplified DNA samples. TCR gene complex appear to be highly conserved. RFLP studies and sequence analysis. Once sequence differences had been characterized, allele specific oligonucleotides were designed to hybridize with the region including the substitution and were used as proves to test PCR amplified DNA samples. TCR gene complexes appear to be highly conserved; RFLP studies and sequence analyses of coding regions reveal limited polymorphism. Examination of the Vβ1 gene revealed only a single nucleotide substitution that resulted in an amino acid substitution located within a hypervariable region of the TCR Vβ1 chain. Furthermore, correlation between Vβ1 RFLP and this sequence difference was not precise. In contrast to the limited polymorphism observed with individual markers, there is considerable polymorphism in the combination of markers inherited together in both TCRα and TCRβ haplotypes. These observations have an important impact upon studies to determine whether TCR genes are associated with disease susceptibility and indicate that studies of families showing a predisposition to a particular disease will be most informative. The inheritance of TCRβ haplotypes was analyzed in families of 40 sibling pairs concordant for the relapsing-remitting form of Multiple Sclerosis (MS). The mean proportion to TCRβ haplotypes identical by descent inherited by MS sibling pairs was significantly increased compared with expected values (means test $p<0.004$) and one allelic form of a TCRβ V region gene segment was overrepresented on MS chromosomes. These results demonstrate that a gene within the TCRβ complex or a closely linked locus influences susceptibility to MS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00525-02-LIG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Human Killer T Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eric O. Long	Visiting Scientist	LIG/NIAID
Others:	Roberto Biassoni	Visiting Fellow	LIG/NIAID
	Mauro Malnati	Visiting Fellow	LIG/NIAID

COOPERATING UNITS *(if any)*

National Institute for Cancer Research, Genoa, Italy (S. Ferrini, A. Moretta, L. Moretta)

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Peripheral blood lymphocytes grown in the presence of high doses of interleukin-2 (IL-2) acquire the ability to lyse tumor cells. This cytolytic activity is not restricted by antigens encoded in the major histocompatibility complex (MHC) and is observed with a variety of both autologous and allogeneic tumors. This activity has been called lymphokine-activated killing (LAK) and has been used in the clinical treatment of tumors. The origin of cells with LAK activity and the mechanism by which they recognize and kill their targets are unknown. Most of the cells with LAK activity display the $CD3^-CD4^-CD8^-CD16^+$ phenotype. Populations of cells with this phenotype were purified by a combination of antibody-mediated complement killing and cell sorting. Clones were also obtained by limiting dilution. RNA hybridizations demonstrated that these cells express only the CD3ε gene of the CD3 complex and the δ gene of the TCR complex. The TCR δ transcripts were derived from unrearranged genes. These observations are consistent with the notion that these MHC-unrestricted cytotoxic cells are derived from an early stage in the T cell lineage. An antibody has been isolated that binds to a cell surface molecule on these cells and that can modulate their cytotoxic activity. A cloning strategy based on cDNA expression is in use to isolate the gene encoding this cell surface molecule.

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LABORATORY OF IMMUNOLOGY
1989 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1988 through September 30, 1989

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It has made rapid progress through the use of new technologies that have created a revolution in immunologic science. These are the techniques of modern molecular biology, monoclonal antibodies, long term lines of cloned normal and transformed lymphocytes, and flow cytometric analysis. These methods combined with newer techniques in protein chemistry and cell biology should allow the solution of many of the major problems that have concerned immunologists and should lead to important advances in efforts to regulate the normal and the disordered immune response.

The Expression of Phosphomyristin C, a Principle Phosphorylation Substrate of Protein Kinase C, is Controlled Developmentally in T Cells and by State of Activation in B Cells

Phosphomyristin C (PMC) is one of the most prominent of protein kinase C (PKC) substrates in B cells. It is strikingly phosphorylated in response to cross-linkage of membrane IgM with anti-IgM antibodies or to treatment of B cells with phorbol myristate acetate (PMA). Not only is PMC an important PKC substrate, it is also the major myristylated protein in B cells and in several other cell types. The degree of expression of PMC, as detected by immunoblotting, is subject to both developmental regulation and regulation by state of activation. Thus, while B cell express and phosphorylate PMC, resting T cells and many T cell lines fail to express detectable PMC. B cell activation, particularly by cross-linking cell surface IgM, not only leads to PMC phosphorylation but causes striking increases in the amount of PMC. By contrast, T cell activation fails to increase PMC levels from their essentially undetectable levels in resting mature T cells. By contrast, immature members of the T cell lineage express abundant PMC. Thus, $CD4^-$, $CD8^-$ thymocytes, the most primitive of T cell populations, express large mounts of PMC; $CD4^+$, $CD8^+$ T cells express considerable PKC, but less than the double negatives. The most mature of thymic T lineage cells, the single positive ($CD4^+8^-$ or $CD4^-8^+$) cells, express barely detectable levels of PMC while peripheral T cells have essentially no detectable PMC. These results suggest that PMC may play an important role in the transmission of membrane receptor generated signals and that the developmental and lineage differences in PMC may be related to striking differences in the response of these cells to environmental stimuli. (P. Hornbeck & W.E. Paul, LI/NIAID; B.J. Fowlkes, LCMI/NIAID; H. Nakabayshi and K.-P. Huang, NICHD; D. Kligman, NIMH).

T Lymphocytes and B Lymphocytes Display Striking Differences in their Regulation of Cytosolic Calcium Concentration

Elevation of cytosolic free calcium concentration $[Ca^{2+}]_i$ is a key element in receptor-mediated activation events in both B and T lymphocytes. Recent advances in the measurement of $[Ca^{2+}]_i$ with calcium-sensing fluorescent probes have made it possible to determine $[Ca^{2+}]_i$ in individual cells in large populations. Using the "second generation" calcium specific fluorescent probe Indo-1, Laboratory of Immunology scientists have examined the responses of both B and T lymphocytes to standard calcium elevating stimuli. Thus, both cell types were exposed to the calcium ionophore ionomycin at concentrations of 0.25 to 0.8 μM. Despite similar uptake of the ionophore, T cells achieved $[Ca^{2+}]_i$ levels five times that of B cells. This greater increase in $[Ca^{2+}]_i$ in T cells is partly accounted for by the more rapid efflux of Ca^{2+} from B cells than T cells, even at when B cell $[Ca^{2+}]_i$ is lower than T cell $[Ca^{2+}]_i$. T cells and B cells also show striking differences in membrane potential as their $[Ca^{2+}]_i$ is elevated. At $[Ca^{2+}]_i$ levels above 300 nM, increased K^+ permeability in T cells results in the hyperpolarization of the T cell. By contrast, B cells which lack the $Ca2^+$-sensitive T cell K^+ channel depolarize when $[Ca^{2+}]_i$ is elevated. When the effect of membrane potential on $[Ca^{2+}]_i$ elevation was examined, it was observed that preventing hyperpolarization by increasing $[K^+]_o$ dramatically reduced the elevation in $[Ca^{2+}]_i$ in T cells and greatly diminished the differences observed between T cells and B cells. These experiments indicate that T cells have potent amplification mechanisms to increase the magnitude of $[Ca^{2+}]_i$ responses. It appears likely that T cells employ such amplification mechanisms to markedly enhance intracellular signalling, possibly because the initial antigen-recognition events may involve only a small fraction of T cell receptors. (Y. Ishida and T.M. Chused, LI/NIAID).

Mapping of the Extent of the Large Duplication of the Rabbit Kappa Light Chain Locus

Rabbits express two distinct isotypic forms of kappa light chains, designated K1 and K2. In general K2 light chains are expressed at much lower levels that K1 light chains. Breeding studies conducted by Laboratory of Immunology scientists and by others have previously shown that the genes for K1 and K2 light chains were linked to one another. Studies are now underway to obtain a physical map of the linkage of these genes by probing DNA digests that have been separted by pulsed field gel electrophoresis or by transverse alternating field electrophoresis. A tentative megabase map has been constructed which indicates a large duplication within a 700 kb region. This region appears to contain two Cκ genes, each with its own Vκ cluster. Work is in progress to test the hyposthesis that low expression of K2 reflects both a functional impairment of the encoded protein structure and an absence of sequences in the Jκ-Cκ intron of K2 that are present in K1 and that are associated with the nuclear matrix. (N. Hole and R. Mage, LI/NIAID)

A Cyclosporine A-Resistant In Vivo Pathway of T Cell Activation

T cell proliferation is a critical element of immune responses to foreign substances; much of that proliferation appears to be mediated by the growth factors IL-2 and IL-4, the production of which is blocked in vitro and in vivo by the action of the immunosuppressive drug Cyclosporine A (CsA). Laboratory of Immunology scientists have been interested in determining the relative importance of this pathway of activation and proliferation in in vivo T cell responses. They had previously observed that that injection of allogeneic lymphocytes into the footpad of a recipient mouse led to striking T cell activation in the draining lymph nodes even in the presence of CsA. Indeed, upon explantation of cells from such animals, the resulting T cell proliferated more vigorously than did cells from animals that had not been treated with CsA. In the past year, evidence has been obtained that indicates that CsA does block IL-2 production in this in vivo system. Thus, sensitive RNase protection assays indicate that no mRNA for IL-2 is produced in the course of responses to allogeneic cells if the animals have been treated with CsA. Nonetheless, the T cells in the CsA-treated mice undergo priming and clonal expansion and demonstration differentiation into precursors of cytotoxic T lymphocytes (CTL). Indeed, upon culture in vitro, the "pre-CTL" develop striking CTL activity and upon rechallenge in vitro develop a response that is indistinguishable in magnitude from that of untreated controls. These results indicate the existence of an in vivo CsA-resistant, IL-2-independent pathway of lymphocyte activation and differentiation that has not yet been described in vitro. Nonetheless, this pathway of activation is insufficient to cause graft rejection, presumably because it does not lead to the development of effector CTL in vivo. However, the observations that IL-2 production and CTL activity can be detected shortly after the cessation of CsA treatment are quite consistent with observations that IL-2 production and CTL activity can often be observed in human and murine allograft models immediately after the termination of CsA therapy. These results suggest the existence of a novel pathway of in vivo T cell activation that may have great significance in normal physiologic immune responses. (G. Pereira and E. Shevach, LI/NIAID).

Structural Regulation of Formation and Surface Expression of Class II MHC Molecules

Class II major histocompatibility molecules are heterodimeric membrane proteins that play a key role in antigen-presentation by binding peptide fragments of antigen and presenting them to antigen-specific T cells. Evidence had previously been obtained that residues in the α and β chains of class II molecules controlled the capacity of these chains to interact pairing of chains encoded in the same genetic region and by the same allelic chromosome favoring (i.e. isotype and allotype specific pairing is favored). During the past year, Laboratory of Immunology scientists undertook an analysis of the mechanisms underlying this preferential expression of cell surface molecules. Through quantitative analyses of surface expression of L cells that had been transfected with two, three or four wild-type class II genes, it was established that the Aβ chain is expressed 3-5 fold more efficiently with the Aα chain than with the Eα chain. Similarly, Eβ is expressed much more efficiently with Eα than with Aα. Indeed, this resulted in the expression of only AαAβ and EαEβ molecules in L cells that had been transfected with genes for all four chains and that expressed balanced production of these chains. However, if there is markedly asymmetric production of individual chains, mixed isotype class II molecules do appear on the cell surface. Studies of allele specific preference in pairing (i.e. the capacity of allelic variants of Aβ chains to pair with a common Aα chain), demonstrated that one important site is contributed to by the residue at position 12 of the βhain. Nonetheless, the results also pointed to other regions of the molecule as playing an important role in determining pairing, with regions located closer to the carboxy terminus having a strong secondary effect. This work has important implications for the means through which class II molecules are assembled and function both in the binding of antigenic peptides and in their interaction with T cell receptors. It also promises to be of considerable importance in understanding the interaction of class II molecules with the accessory structure CD4. (A. Sant and R. Germain, LI/NIAID).

An H-2K^b Peptide Inhibits the Recognition of H-2K^b by Specific T Cells

The interaction between T cells and their receptors has been difficult to study because both the receptor and the ligand are cell surface molecules. Laboratory of Immunology scientists have developed soluble form of class I MHC molecules in order to study these interactions. A soluble analog of the H-2K^b (K^b) protein has been shown to partially block the response K^b-specific T cells to their targets. When a target cell bearing a mutant form of K^b (K^{bm10}) was used, the soluble K^b was a more effective inhibitor, suggesting that residues that were mutated in the K^{bm10} participated in the binding event. To examine the nature of the interaction more thoroughly, a peptide was prepared that included the region of the K^b molecule that was mutated in K^{bm10}. This peptide, involving residues 163-174 inhibited the response of a K^b-specific hybridoma, with half-maximal inhibition requiring a peptide concentration of 10^{-4}M. Experiments in which both the target cell and the T cell were pre-pulsed with the peptide indicated that inhibition was achieved by binding to a receptor on the T cell. It was however observed that 163-174 was also an effective inhibitor of cytotoxic T cells specific for mutant K^b molecules other than K^{bm10}, apparently involving contact antigenic residues quite distinct from 163-174. For that reason, it has been proposed that the peptide interferes with a common "docking" function and that the region 163-174 is important in the interaction of T cells and the K^b class I molecule in a general rather than specific manner. This, in turn suggests that the interaction of T cell receptors with class I molecules is a complex one involving both shared and unique portions of the class I molecule. (J. Schneck & D.H. Margulies, LI/NIAID; T. Munitz & A. Singer, EIB/NCI).

A Transgenic Mouse Expressing a Soluble Form of H-2D^d Can Develop Cytotoxic T Cells Specific for H-2D^d

The induction of immunologic unresponsiveness of T cells to autologous antigens, particularly to class I and class II MHC molecules, is central to self/non-self discrimination in the immune system. In order to examine the requirements for establishment of immunologic tolerance to the class I molecule H-2D^d (D^d), a transgenic mouse was produce that expresses D^d as a secreted but not a cell surface protein. The transgene consisted of a construct involving a1 and a2 domains (the principal polymorphic regions of class I molecules) of D^d and the carboxyterminal domains of Q10^b, an obligatorily secreted class I molecule. The resulting product of the transgene is the secreted protein designated D^d/Q10^b The transgenic mouse expressing this gene is designated C57BL/6tD^d/Q10^b. These mice express five to ten copies of the transgene located at a single autosomal locus. The soluble class I molecule encoded by the transgene is found in the serum at a concentration of 100 mg/ml but no detectable cell surface D^d was found. Strikingly, primary cultures of spleen cells from these mice were capable of developing cytotoxic T lymphocytes specific for membrane H-2D^d. These results indicate that soluble class I molecules are not effective inducers of immunologic tolerance, at least among precursors of cytotoxic T lymphocytes and indicate that cell surface expression is a key requirement for the induction of the tolerant state for MHC class I antigens. (R. Hunziger and D. Margulies, LI/NIAID).

Mapping of the Major HIV gp160 Epitope Recognized by Cytotoxic T Lymphocytes From H-2^d Mice

In an effort to understand the molecular and cellular regulation of T cell immunity to HIV proteins, Laboratory of Immunology scientists and their colleagues have developed a model system for testing the immunogenicity of HIV proteins and used it to identify dominant T cell epitopes and to determine the effect of HIV sequence variation on recognition of such epitopes. The env gene encoding the gp160 molecule of the IIIB isolate of HIV-1 was inserted into a vaccinia vector. Purified stocks of the resultant virus were used to immunize mice. Stimulator and target cells were prepared by transfecting BALB/c-3T3 and L cells with the env gene in an SV-40-driven expression vector. Long term T CD8+ cell lines and clones specific for gp160 were generated in this manner and studied for their specificity. Cytotoxic T cells obtained from H-2^d mice were screened for their recognition of a set of peptides derived from the gp160-IIIB sequence. Only a single peptide, corresponding to positions 315-329, sensitized target cells for attack by the gp160-specific CTL. The recognition of the 315-329 peptide required D^d as a restriction element. This site appears to be the major epitope recognized by H-2^d CTL generated after only a single in vitro immunization. This site is one of substantial sequence variation among HIV isolates. Thus, gp160-IIIB-specific CTL constitute a distinct and non-overlapping set of CTL from those that recognize gp160-MN even though the same region of gp160 constitutes the major epitope in both cases and both responses utilize D^d as a restricting element. Furthermore, gp160-RF is not immunogenic with seven distinct class I molecules and is not recognized by CTL generated in response to either gp160-IIIB or MN. These results indicate that T cell epitopes in HIV gp160 are a site of sequence variation and suggest that they may be subject to strong selection in vivo by the action of antigen-specific CTL. (R. Germain, LI/NIAID; B. Moss, LVD/NIAID; H. Takahashi and J. Berzofsky, MB/NCI).

Cloning of a Novel Type II Integral Membrane Protein

The response of T lymphocytes to foreign substances is a complex process that requires the participation of a series of surface proteins. During the past year, Laboratory of Immunology scientists have obtained a cDNA clone for one such protein that had been identified with the monoclonal antibody A1. A1 cDNA was isolated through the use of an expression cloning system involving immunoselection of COS 7.2 cells that had been transfected with T cell derived cDNA in the eukaryotic expression vector CDM8. The sequence of the A1 cDNA resulted in a deduced polypeptide consisting of 262 residues, multiple cysteines and 3 potential N-linked glycosylation sites. A1 has the features of a type 2 integral membrane protein, with it carboxyterminus exposed extracellularly and its aminoterminus in the cytoplasm. It displayed significant homology with the extracellular domains of several other type II proteins, particularly with FcεRII, the low affinity IgE receptor also designated CD23. The similarity of A1 to FcεRII and to asialoglycoprotein receptors suggests that it may function as a cell surface ligand, possibly as a receptor for the Fc portion of an Ig. Furthermore, Southern analysis suggests that A1 is a member of a multigene family. Efforts to isolate the members of this family and to obtain information regarding their functional significance is now in progress. The availability of a cDNA clone for A1 promises to have great impact in the unraveling of the mechanisms through which T cells receive information from extracellular sources. (W. Yokoyama and E. Shevach, LI/NIAID; D. Cohen, LCB/NIDDKD).

IL-2 is Required for Production of IL-4 by Resting T Cell Populations

T cell populations produce two potent growth promoting polypeptides, IL-2 and IL-4. In vitro studies employing long term T cell clones have indicated that IL-2 is produced by one set of T cell clones (T_{H1} cells) while IL-4 is produced by an alternative set of such clones (T_{H2} cells). Examination of normal lymphoid cell populations using a highly sensitive and specific IL-4 bioassay indicate that resting T cell from normal mice make very limited amounts of IL-4 in response to stimulation with anti-CD3 antibody adsorbed onto the surface of a culture well. By contrast, large T cells from normal mice and, to a considerably greater degree, large T cells from mice that have been infected with N. brasiliensis or immunized with anti-IgD antibodies, make striking amounts of IL-4 in response to anti-CD3 stimulation. The response of resting T cells from normal mice is markedly enhanced by the addition of IL-2 to the culture and the response is completely blocked by the addition of anti-IL-2 antibody which neutralizes the IL-2 produced in situ. This result indicates that production of IL-4 by resting T cells is IL-2 dependent. By contrast, large T from immunized animals make substantial amounts of IL-4 without the addition of IL-2 and this response is only partially inhibited by anti-IL-2 antibody, indicating that immunized animals contain a substantial population of T cells that have developed the capacity to produce IL-4 in response to receptor cross-linkage without need for further IL-2-mediated differentiation events. IL-2-independent IL-4 producing cells ("activated T cells") can be generated from the resting normal T cell population by culturing these cells for five days with plate-bound anti-CD3 antibody and both IL-2 and IL-4. These results indicate that the capacity to produce IL-4 is a highly regulated property and that IL-4 producing cells can be generated in substantial numbers from resting populations but they require the action of existing lymphokines to complete this differentiation event. This information is of considerable importance in clarifying the factors that determine the type of immune response which emerges in response to various immunization protocols and may allow the development of methods to tailor the immunization protocol to the biologic response desired. (S.Z. Ben-Sasson, G. LeGros, and W.E. Paul, LI/NIAID; D. Conrad, LI/NIAID & Med. Coll. Va.; F.D. Finkelman, USUHS).

Cyclosporine A Inhibits Granule Exocytosis in Cytotoxic T Cells and in Rat Basophil Leukemia Cells

Cyclosporine A (CsA) is a potent immunosuppressive agent known to interfere with the transcription of lymphokine genes. Although much of the immunosuppressive action of CsA appears to results from inhibition of lymphokine production, it is now clear that the drug inhibits other functions that are very important in immune responses. Laboratory of Immunology scientists have observed that CsA partially inhibited lysis of antigen-bearing target cells by cytotoxic T lymphocytes (CTL). In the course of the cytolytic event, T cells release the contents of intracellular granules that contain proteins believed to play an important role in the effector function of CTL. CsA markedly inhibited granule exocytosis induced by interaction of CTL with either target cells or with antibodies to the T cell receptor. Furthermore, CsA inhibited granule release caused by phorbol esters and calcium ionophores, suggesting that CsA mediated its inhibition not by blocking either $[Ca^{2+}]_i$ elevation or activation of protein kinase C but rather by acting on signalling events distal to these steps. In keeping with that, CsA had no inhibitory action on increase in phosphatidyl inositol metabolism in CTL as a result of receptor cross-linkage with anti-T cell receptor antibody. Furthermore, the inhibitory effects of CsA do not appear to involve the cAMP-dependent protein kinase inhibitory pathway nor does CsA have any effect on the Ca^{2+}-induced binding of calmodulin to calmodulin-binding proteins. CsA inhibition of granule exocytosis was also observed in rat basophil leukemia cells. CsA had no effect on IgE receptor-mediated phosphatidyl inositol hydrolysis but caused a 90% inhibition in serotonin release. These results indicate that CsA is a potent inhibitor of receptor-mediated granule exocytosis by cells of the immune system. They suggest that such effects may be an important component of the immunosuppressive action of CsA and may provide useful tools for the study of exocytosis in general. (G. Trenn, R. Taffs, E.M. Shevach and M. Sitkovsky, LI/NIAID).

Interleukin-4 is Produced By Mast Cell Lines and by Splenic Non-B, Non-T Cells in Response to Fc Receptor Cross-linkage

Interleukin-4 (IL-4) is a member of a set of lymphokines that are encoded by genes that are linked to one another on chromosome 11 of the mouse and on the long are of chromosome 5 in the human. The other members of the "IL-4 family" of lymphokines are interleukin-5 (IL-5), interleukin-3 (IL-3) and granulocyte-macrophage colony stimulating factor (GM-CSF). IL-4 and the other members of this family are produced by a set of long term mouse T cell lines designated TH2 cells. Because of the properties of the lymphokines, TH2 cells appear too be particularly important in controlling humoral immune responses and in the mediation of allergic inflammation. Laboratory of Immunology scientists and their colleagues have now demonstrated that long term, IL-3-dependent mast cell lines produce IL-4 and the other members of the IL-4 family of lymphokines as well as interleukin-6 (IL-6) upon exposure to calcium ionophores or upon cross-linkage of their high affinity Fcε receptor. For example, the long term mast cell line CFTL-12 shows striking stimulation in the production of IL-4, IL-3, GM-CSF and IL-6 in response to the calcium ionophore ionomycin. This is associated with very striking induction of mRNA for these lymphokines and for IL-5. Similarly, sensitization of the high affinity Fcε receptors of these cells with a monoclonal IgE anti-DNP antibody prepares these cells to secrete lymphokine and to elevate levels of lymphokine mRNA upon the addition of polyvalent DNP-proteins or anti-IgE antibodies. These cells also release histamine in response to such stimuli and the requirements for histamine release and for lymphokine production is essentially the same. The lymphokines produced by these cells, IL-3, IL-4, IL-5, GM-CSF and IL-6, but not interleukin-2 (IL-2), intereferon γ (IFNγ) or lymphotoxin (LT), are well suited to mediate allergic inflammatory responses, which strongly suggests that mast cells may participate in allergic responses not only by producing acute mediators of immediate hypersensitvity but also by producing lymphokines that regulate the growth and differentiation of many cell types that make active contributions to the allergic inflammatory state. (M. Plaut, LI/NIAID and Johns Hopkins School of Medicine; W.E. Paul, LI/NIAID; J. Pierce, R. Nordan and J. Hanley-Hyde, NCI).

Recently, it has been observed that a population of non-B, non-T cells in the spleen and bone marrow of non-treated mice can produce substantial amounts of IL-4 in response to stimulants that cross-link Fcε and Fcγ receptors. This response is also dependent on the presence of IL-3. The action of IL-3 appears to involve the differentiation of non-B, non-T cells to develop into lymphokine producing cells. A similar effect can be achieved either by treating mice with IL-3, in which case the non-B, non-T cells can produce IL-4 in response to plate-bound IgE or IgG2a, or by immunizing the animal by infection with the nematode Nippostrongylus brasiliensis or by treatment with anti-IgD antibodies. The nature of the cell that produces IL-4 in response to Fc receptor cross-linkage is not known but it does not appear to be either an NK cell or a macrophage. It may well constitute a member of the mast cell lineage or cells of the myeloid line. This result indicates that a normal non-T cell population has the capacity of producing members of the IL-4 lymphokine family and suggest that these non-B, non-T cells may have an important physiologic role in amplifying immune responses and amplifying the cellular component of Fc receptor-mediated allergic inflammation. (S.Z. Ben-Sasson, G. LeGros, and W.E. Paul, LI/NIAID; D. Conrad, LI/NIAID & Med. Coll. Va.; F.D. Finkelman, USUHS).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in the U.S. and international immunological communities. They serve on editorial boards of many scholarly publications. Dr. Ethan Shevach is editor-in-chief of the Journal of Immunology, and is a member of the editorial boards of Cellular Immunology, of the Journal of Immunological Methods and of Current Protocols in Immunolgy and is a member of the Council of Biology Editors.

Dr. William Paul is the editor of the Annual Review of Immunology and is an advisory editor of the Journal of Experimental Medicine, an associate editor of Cell, a member of the editorial boards of Immunological Reviews and of the Journal of Molecular and Cellular Immunology and a transmitting editor of International Immunology. He completed a term as consulting editor for immunology of the Cecil Textbook of Medicine. Dr. Paul edited the second edition of Fundamental Immunology which appeared this year. The first edition, also under Dr. Paul's editorship, achieved the position as the leading textbook of advanced immunology.

Dr. Ronald Germain is a deputy editor of the Journal of Immunology and is a member of the advisory editorial board of the Journal of Molecular and Cellular Immunology and of the Annuales de l'Institut Pasteur. Dr. Rose Mage is a member of the editorial board of Immunogenetics. Dr. John Inman is a member of the editorial board of Analytical Biochemistry and is an advisory editor of Molecular Immunology. Dr. Thomas Chused is a member of the editorial board of Cytometry.

Dr. Paul is a member of the Board on Basic Biology of the National Research Council and this board's Committee on Research Opportunities in Biology. Dr. Paul is a member of the committee to visit the Division of Medical Sciences of the Board of Overseers of Harvard College; of the advisory committee of the Pew Scholars Program in the Medical Sciences; and of the selection committee for the RJR/Nabisco Scholars Award in Immunology. He is a member of the scientific review board of the Howard Hughes Medical Institute, of the board of scientific advisors of the Jane Coffin Childs Fund for Medical Research, of the board of scientific consultants of the Memorial-Sloan Kettering Cancer Center; of the scientific advisory committee of the Cancer Research Institute, Inc.; of the Research Visiting Committee of the State University of New York Health Science Center at Brooklyn; and of the board of directors of the Foundation for Advanced Education in the Sciences. He is chariman of the advisory committee of the Harold C. Simmons Arthritis Research Center.

During the past year, Dr. Paul was the "Once-a-Year" Lecturer at the Departments of Cell Biology and Chemical Immunology of the Weizmann Institute of Science, Rehovot, Israel; was the Nelson Medical Lecturer at the University of California School of Medicine at Davis; and presented the Graduate School Lecture at the State University of New York Health Sciences Center at Brooklyn. Dr. Paul was co-organizer of the Colloquim on Immunology and Diabetes, sponsored by the National Institute of Diabetes, Digestive and Kidney Diseases, in Bethesda, MD; chaired a session at the New York Academy of Sciences Symposium on Interleukin-6; and chaired the symposium on "Cell Cooperation" at the Seventh International Congress of Immunology in Berlin. He presented the Concluding Lecture at the Sixth International Lymphokine Workshop in Evian, France, and was an invited lecturer at the Banbury Center Conference on Manipulating the Immune Response, at Cold Spring Harbor, NY; at the Third Annual Conference on Clinical Immunology of the Clinical Immunology Society, in San Francisco, CA; at the International Mochida Memorial Symposium in Tokyo, Japan; at the symposium "Molecular Biology of Human Cells", celebrating the fifth anniversary of the Institute for Molecular and Cellular Biology of Osaka University; in the American Academy of Allergy and Immunology Postgraduate Education Course in San Antonio, TX; at the European School of Oncology Symposium on Regulation of

Cell and Tissue Function by Peptide Growth Factors, in Venice, Italy; at the University of Indiana Symposium "Windows to the Future" at Indianopolis IN; and at the International Conference on Molecular Aspects of Immune Responses and Infectious Diseases in Rome, Italy.

Dr. Shevach was elected to membership in the Association of American Physicians. He is a member of the Scientific Advisory Board of the American Leprosy Foundation (Leonard Wood Memorial). Dr. Shevach was a visiting professor at the Cancer, AIDS and Immunology Research Institute of Bar-Ilan University in Israel and as an invited speaker at the Lake Tahoe Conference on Lymphocyte Receptors and Signalling.

Dr. Germain received the USPHS Superior Service Award. He was an invited lecturer at the 19th Princess Takamatsu Symposium on Cancer, in Tokyo, Japan; was an invited lecturer at the Immunex-University of Washington Guest Scientist Series, Seatle, WA; was an invited lecturer at the annual meeting of the American Society of Nephrology; and was a plenary lecturer at the UCLA meeting on Immunogenicity.

Dr. Mage serves as the American Association of Immunologists' representitive to the board of the American Type Culture Collection (ATCC). She is a member of the Board of Directors of the ATCC and was elected to its Executive Committee. She was also elected to membership on the Board of Directors of the Foundation for Advanced Education in the Sciences. She is a Fellow of the American Association for the Advancement of Science.

Dr. David Margulies was an invited speaker at the Minisymposium on "Class I Molecules: Strucutre/Function" at the Annual Meeting of the American Association of Immunologists in New Orleans, LA and at the Jackson Laboratory meeting on Transgenic Mice and Mutants in MHC Research, in Bar Harbor, ME. He is a member of the Immunology and Immunotherapy Advisory Committee of the American Cancer Society.

Dr. Michail Sitkovsky gave an invited lecture in the Distinguished Investigator Program at the Mayo Medical School in Rochester, MN. He was a speaker at the Symposium on Cytotoxic Lymphocytes held at the DNAX Institute of Molecular Biology in Palo Alto, CA and was the chairman of the workshop on "Mechanisms of T-cell Mediated Cytotoxicity" at the Seventh International Congress of Immunology in Berlin.

In addition, Laboratory of Immunology scientists presented seminars at major universities, medical schools and research institutes in the United States and abroad.

Administrative, Organizational and Other Changes

During the past year, the Laboratory of Immunology appointed Dr. Michael Lenardo to a "tenure track" investigator position. Dr. Lenardo was chosen from a large group of individuals identified through a national search for young molecular immunologists interested in cell biological problems. He came to the Laboratory after completing a successful post-doctoral training period at the Whitehead Institute and MIT under the supervision of Dr. David Baltimore. Dr. Lenardo will bring strengths in many aspects of molecular biology to the Laboratory, with particular emphasis on regulation of gene transcription. Dr. Richard Asofsky transferred to the Laboratory of Immunology as Head of the Section of Experimental Pathology.

The Laboratory continues to be a major training center for youg immunologists. During the past year several individuals completed post-doctoral training or sabbatical periods in the Laboratory of Immunology. Among these were Geroffrey Chazen, Dan Conrad, Robert Lazar, Steven Maxfield, Junichi Ohara, Geraldo Pereira, Marshall Plaut, David Presky, Franca Ronchese, Andrea Sant, and Jonathan Schneck. Each of these scientists made important contributions to the Laboratory of Immunology research program.

During the past year, several scientists joined the Laboratory of Immunology for post-doctoral training or sabbatical visits. They included Wolf-Henning Boehncke, Jean-Louis Boulay, Charles Chu, Maripat Corr, George Dos Reis, Wen-Jing Fang, Yehoshua Gozes, Achsah Keegan, Rolf Koenig, Jacqueline Little, John Meligeni, Luigi Racioppi, Randall Ribaudo, Robert Seder, Mariangela Segre, Toshio Tanaka, Debra Weinstein, and David Wilde. It is anticipated that they will continue a tradition of excellence established by a long a series of outstanding trainees in the Laboratory.

The Laboratory wishes to particularly note the retirement of Mr. William Humphrey after thirty-eight years of governemnt service, of which more than twenty were in the Laboratory of Immunology. Mr. Humphrey was a key colleague in the research program headed by Ms. Rose Lieberman that achieved international recognition in the field of immunoglobulin genetics. After Ms. Lieberman's retirement, Mr. Humphrey was am important factor in the rapid establishment of Dr. Michail Sitkovsky's research program in the Laboratory of Immunology. His efforts represent an outstanding contribution to the success of LI and NIAID research programs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00030-21LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	William E. Paul	Chief	LI, NIAID
Others:	P. Hornbeck	Senior Staff Fellow	LI, NIAID
	M. Plaut	Guest Researcher	LI, NIAID
	A. Keegan	NRC Fellow	LI, NIAID

COOPERATING UNITS (*if any*)

NICHD (K. P. Huang); NCI (W. Tsang)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.65	1.25	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
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☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Activation of cells of the immune system usually requires a ligand-receptor interaction that results in cross-linkage of cell surface receptors. In B cells and T cells, the receptors are membrane immunoglobulin (Ig) and the T cell receptor (TCR), respectively. The cross-linkage of these surface molecules leads to activation of the inositol phospholipid metabolic pathway and to activation of protein kinase C, with subsequent phosphorylation of a series of kinase substrates. One very striking substrate is phosphmyristin C (PMC), which is the major myristylated protein in B lymphocytes. PMC is strikingly phosphorylated in B cells and B lymphomas in response to cross-linkage of membrane Ig or to treatment with phorbol myristate acetate (PMA). Furthermore, stimulation of resting B cells with anti-Ig antibody causes striking increases in levels of expression of PMC. By contrast, mature T cell express little or no PMC but immature thymocytes, lacking CD4 and CD8, are rich sources of PMC. The developmental control of PMC expression, its upregulation upon B cell activation and its high level of myristylation suggests it may play a major role in mediation of receptor-mediated signalling events.

Another aspect of immunologic recognition is the binding of antigen by IgE antibodies bound to Fcε receptors on mast cells. Long term mast cell lines sensitized with monoclonal IgE anti-DNP antibodies show striking increases in mRNA for a series of lymphokines including IL-3, IL-4, IL-5, GM-CSF and IL-6. This event requires receptor cross-linkage and has dose response relationships similar to those required for histamine release by such cells. The capacity of long term mast cell lines and of normal non-B, non-T cells to produce lymphokines is determined by the presence of IL-3, indicating that IL-3 induces a differentiative step that regulates receptor-mediated stimulation of lymphokine production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00035-14 LI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Specificity in Immune Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Inman	Section Head	LI, NIAID
Others:	A. Lees	Special Volunteer	LI, NIAID
	P. Highet	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*
Dept Medicine, USUHS (F. Finkelman, J. Mond, M. Brunswick); Dept Biophysics, Johns Hopkins School of Medicine (H. M. Dintzis)

LAB/BRANCH
Laboratory of Immunology

SECTION
Bioorganic Chemistry Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major part of this project is directed toward the design and synthesis of highly specific and efficient immunomodulators. Effector molecules (e.g. monoclonal antibodies directed against designated cell surface proteins) are multiply and covalently linked to soluble polymer carriers of high molecular weight using heterobifunctional reagents. In some studies, protein-protein heteroconjugates have been prepared. New crosslinking reagents have been designed, synthesized and used as part of this project. Special emphasis has been given to improving the chemistry for functionalizing antibody molecules through their polysaccharide moieties and to the cross-linking of these derivatives to functionalized carriers. Collaborative studies employing the immunomodulators have dealt largely with a variety of cell surface factors involved in activation, growth and differentiation of B lymphocytes. For example, it has been shown that anti-IgD alone delivers a weak mitogenic signal to resting B cells, but when it is multiply linked to a soluble polymer such as dextran of MW $\geq$ 200,000, strong mitogenic stimulation occurs at antibody concentrations ranging from 0.01 to 1.0 ng/ml. Collaborative studies have shown that, throughout most of this range, no perceptible increases in intracellular ionized calcium occurred. Yet, these conditions of stimulation are believed to closely approximate normal, T cell-independent antigenic signalling.

Another important objective of this project is to explore the property of general multispecificity of antibodies and other receptors. A study has been completed that measured the distribution of binding constants of a monoclonal antibody for a large collection of diverse compounds (not related to the homologous immunizing hapten). The reults strongly support the statistical basis for multispecificity. Quantitative affinity chromatography was used to measure binding constants down to very small values. Techniques for preparing specialized affinity adsorbents were developed for these and other studies. Further work is planned for exploring alternative modes of complementation of ligands in receptor sites and relating these findings to specificity in immune systems and networks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00036-24 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	B. Newman	IRTA Fellow	LI, NIAID
	N. Hole	Visiting Fellow/Associate	LI, NIAID
	M. Allegrucci	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)* LM, NIDR (N. McCartney-Francis); LOM, NIDR (N. Harindranath); Basel Inst. Immunol., Basel, Switzerland (A. S. Kelus); Microbiol., Loyola Stritch Sch. Med. Chicago, (K. Knight); Dept Biochem., Univ. Geneva, Switzerland (J. -C. Jaton); Natl. Univ. Mexico, Mexico City, Mexico (E. Lamoyi)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

We use techniques of classical immunogenetic and of molecular biology to study the genetics of rabbit immunoglobulins (Igs) and T cell receptors and the regulated expression of the genes that encode them during lymphoid cell development.

Oligonucleotides corresponding to two functional Jκ genes and to a region of K1b9 sequence that differs from the highly similar K2 gene, were designed and used to amplify a postulated abberrant b9 message in Basilea mutant rabbits using Polymerase Chain Reaction (PCR) after reverse transcription of the mRNA. The sequences obtained show the normal spliced product in mRNA from control wild-type rabbits and demonstrate that the postulated alternative splice site was used in mRNA from the mutant rabbits.

The sequence (4550 bases) of a genomic 6 kb Cβ Eco RI fragment from an animal of haplotype b was shown to represent the rabbit homolog of Cβ2 genes in other species. Comparison of the exon sequences in this genomic DNA from haplotype b with those in a Cβ2 cDNA from haplotype a, revealed replacement changes in exons 1 and 3 that probably represent allotypic forms of the rabbit Cβ2 genes.

Some rabbits are unusual in having three different copies of Cβ genes (Cβb haplotype) unlike others of Cβa haplotype that have only two. The second gene on a 14 kb Eco RI fragment appears to be a chimeric genomic Tcr β chain gene that may have arisen by an unequal crossing over event analogous to that which may have deleted Cβ1, Dβ2 and Jβ2 in NZW mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00134-27 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Richard Asofsky, M.D.	Section Head	LI, NIAID
Others:	J. Little, Ph.D.	IRTA Fellow	LI, NIAID
	S-I. Minato, M.D.	Visiting Fellow	LI, NIAID
	R. Lazar, M.D.	Medical Staff Fellow	LI, NIAID
	Y. Gozes, Ph.D.	Visiting Associate	LI, NIAID
	A. E. Brooks	Biological Tech.	LI, NIAID
	D. F. Amsbaugh	Biologist	LI, NIAID
	T. M. Cox	Coop Student	LI, NIAID
	M. Segre, Ph.D.	Guest Scientist	LI, NIAID

COOPERATING UNITS *(if any)*

Infectious Diseases Unit, Boston City Hospital (C. Sulis, MD); Bioquest Co., Rockville, MD (H. Kun-Chu, MD)

LAB/BRANCH

Laboratory of Immunology

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER.
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various mitogens (LPS, Dextran Sulfate, anti-immunoglobulin, and Salmonella mitogen) produce terminal differentiation and immunoglobulin secretion in B lymphoma and B lymphocyte somatic cell hybrids. There is evidence that LPS with a small peptide contamination is a much more effective inducing agent than highly purified LPS. TH 2.54 is a hybrid which is induced by several agents (LPS, anti-Ig, phorbol esters) to secrete both IgM and IgG2a. Secretion of IgG2a is increased without concomitant lowering of IgM secretion by simultaneous exposure to recombinant γ-interferon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00224-08 LI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monoclonal Antibodies as Probes for T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	W. Yokoyama	Senior Staff Fellow	LI, NIAID
	G. Chazen	Medical Staff Fellow	LI, NIAID
	B. Classen	Staff Fellow	LI, NIAID
	K. Roberts	Visiting Associate	LI, NIAID
	G. Pereira	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*
Dept of Dermatology, University of Vienna, Vienna, Austria (G. Stingl); Biological Resources Branch, NIAID, NIH (J. Coligan); Laboratory of Chemical Biology, NIDDKD, NIH (D. Cohen)

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	6.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objectives of our studies are to characterize cell surface structures on T lymphocytes and secreted lymphocyte products which play critical roles both in vivo and in vitro in the process of lymphocyte activation. We have adapted a eukaryotic expression system for cDNA cloning of cell surface antigens by selecting cells which have been transfected with a cDNA library with monoclonal antibodies (mAbs). A cDNA which encodes a murine cell surface, disulfide-linked 85 kD dimer, which had been identified with several mAbs was cloned by this approach and shown to be a member of a novel polymorphic, supergene family of Type II integral membrane proteins. cDNA sequencing studies of the T cell receptor γδ-chains express by Dendritic Epidermal T cells (DETC) have demonstrated that the pairing of the TCR γ- and δ-chains is restricted, but that significant diversity is still generated in the CDR3 region of the δ-chain. A comprehensive analysis of the relationship between the expression of a given antigen encoded by the Ly-6 multigene family and its reactivity with a given anti-Ly-6 mAb was performed by transfecting multiple Ly-6 cDNAs into COS cells and studying the reactivity of the transfectants with a large panel of anti-Ly-6 mAbs.

Our analysis of production by and responsiveness to lypmhokines of DETC cell lines have shown that these lines have novel cytokine requirements for the induction of proliferation, that their autocrine may be mediated by previously uncharacterized T cell growth factors, and that the spontaneous production of cytokines by several of these lines can be down-regulated by anti-CD3. We have shown that the newly cloned cytokine, IL-7, in combination with phorbol esters is capable of acting as a T cell growth factor for resting T cells. Lastly, we have identified a new, potentially major pathway of IL-2-independent T cell activation which functions during the in vivo response to alloantigens and which is resistant to the immunosuppressive drug, Cyclosporine A (CsA).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00226-08 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	B. Newman	IRTA Fellow	LI, NIAID
	N. Hole	Visiting Fellow/Associate	LI, NIAID
	M. Allegrucci	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LOM, NIDR (N. Harindranath); Basel Inst. Immunol. Basel, Switzerland (A. S. Kelus); Dept Microbiol. Loyola Stritch Sch. Med. Chicago, (K. Knight); Dept Biochem., Univ. Geneva, Geneva, Switzerland (J. -C. Jaton); Natl Univ. Mexico, Mexico City (E.Lamoyi)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒X(c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study genes of the rabbit immune system by techniques of molecular biology. A simple technique for analysis of restriction fragment length polymorphisms (RFLP) using small numbers of cells from sources such as sperm and peripheral blood, was used to determine recently discovered RFLP types in the Jκ region of animals previously studied for Tcr β and Vκ and Cκ RFLP types. Absence of linkage to Cβ and complete linkage to previously reported Vκ and Cκ markers was observed. The Jκ RFLP types were also used to distinguish the Jκ2 and Jκ1 clusters in mapping studies of the complex duplicated κ region of the rabbit. A tentative megabase map has been constructed which suggests that the duplicated Cκ genes are within a 700 kb region. There are two Cκ genes each having their own Jκ cluster. Our data suggest that there are two different Vκ clusters each associated with a Cκ gene. The closest Vκ gene to the Cκ2 is within a 90 kb fragment. Thus, the defect in expression of the K2 gene may not be explained simply in terms of relative distance from a Vκ cluster.

In order to more precisely localize the cross-over sites in three VH-CH recombinants, we have used Southern blot analyses and a panel of specific genomic and cDNA probes spanning the rabbit heavy chain locus. The results indicate that each recombination point was 3' of the entire VH region and 5' of JH. Probes that identify DH genes located between VH and CH are further delineating the sites of the recombinations.

The F-I recombination may have a relationship to the mutation that generated the Alicia rabbit strain which shows an atypical VHa2 phenotype; the mutational event that caused this arose in an animal carrying the F-I recombinant chromosome and may be acting in conjunction with this recombinant to alter VH gene expression. Whereas Southern analyses revealed RFLP throughout the region analysed in different haplotypes (see above), F-I and ali F-I are thus far indistinguishable. Thus with the technique used, no major alterations, deletions or insertions are detectable. Now megamapping by PFGE analyses has revealed a difference that is detectable using a probe from the 3' end of the VH region. The fragments are more than 100kb in size and differ by about 15 kb.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00349-07 LI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	A. Sant	Special Volunteer	LI, NIAID
	F. Ronchese	Visiting Associate	LI, NIAID
	G. Otten	Special Volunteer	LI, NIAID
	L. Hendrix	Research Technician	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS (if any)
LIG, NIAID (E. Long); LCMI, NIAID (R. Schwartz)

LAB/BRANCH
Laboratory of Immunology

SECTION
Lymphocyte Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.75	2.25	1.50

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Class II MHC (Ia) gene products play critical roles in a variety of T lymphocyte responses. A combination of immunological, molecular genetic, and biochemical approaches is being used to study the relationship between Ia structure and function.

Ia expression has been examined following transfer of class II genes into L cells. These experiments have shown that the efficiency of cell-surface Ia expression is controlled by both allele-specific and isotype-specific structural features of the α and β chains. There are strong quantitative preferences in the formation and transport of certain heterodimers, explaining the phenotype of cells expressing multiple class II MHC α and β chains. In vitro mutagenesis studies have mapped two major regions within the Ia molecule that play key roles in controlling the conformation of Ia molecules required for their transport to the cell membrane.

Analysis of various recombinant Ia molecules with monoclonal antibodies have revealed an unexpected asymmetry in the conformational flexibility of the postulated α-helical regions of the polymorphic Ia domains. Site-directed mutagenesis studies have mapped the precise role of polymorphic residues of the I-E molecule with respect to T cell receptor interaction or peptide antigen binding.

These studies are providing new insight into the rules governing T cell co-recognition of antigen and MHC molecules, and the biochemistry and cell biology of protein multimer synthesis and transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00394-06 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. H. Margulies	Section Head	LI, NIAID
Others:	L. Boyd	Chemist	LI, NIAID
	C. Hoes	Biologist	LI, NIAID
	J. Schneck	Medical Staff Fellow	LI, NIAID
	R. Hunziker	Guest Worker	LI, NIAID
	S. Kozlowski	Medical Staff Fellow	LI, NIAID
	R. Ribaudo	Staff Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LI, NIAID (G. Otten, R. Germain, W. Tsang, W. Paul); MB, NCI (H. Takahashi, J. Berzofsky); BRB, NIAID (W. L. Maloy, J. Coligan); EIB, NCI (T. Munitz, A. Singer)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	4.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ongoing focus of the laboratory is to understand in detail the structure/function relationships of cell surface molecules involved in the immune response, employing a combination of methodologies that include molecular biological manipulation of cloned major histocompatibility complex genes, expression analysis of those genes following transfection in various tissue culture cells, expression analysis of those genes following introduction into the germ line of various mouse strains, and functional analysis of purified protein derived from molecularly engineered soluble analogues of these histocompatibility genes. Significant progress over the past year has been achieved in: a) evaluating the in vitro biochemical requirements for stimulation of an alloreactive T cell hybridoma in particular with respect to the structure of the α3 domain of the class I MHC molecule; b) an estimate of the affinity of an anti-H-$2K^b$ T cell receptor for H-$2K^b$; c) the identification of amino acid residues 163-174 of the H-$2K^b$ protein as a potential docking site for a significant proportion of anti-H-$2K^b$ T cell receptors; d) analysis of the biochemical and cellular requirements for the generation of self-tolerance to cell surface molecules using a transgenic mouse system as a model; e) development of systems for studying the interaction of class I molecules with in vitro translation and posttranslational assembly of MHC class I molecules to antigenic peptides; f) the localization of amino acid residues of the H-$2D^d$ molecule critical to the interaction with an HIV derived, H-$2D^d$ restricted antigenic peptide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00403-06 LI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. N. Germain	Section Head	LI, NIAID
Others:	T. Saito	Visiting Associate	LI, NIAID
	C. Layet	Visiting Fellow	LI, NIAID
	M. Okajima	IRTA/Special Volunteer	LI, NIAID

COOPERATING UNITS *(if any)*
FDA (L. Matis)

LAB/BRANCH
Laboratory of Immunology

SECTION
Lymphocyte Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T lymphocytes divide and differentiate when they receive an appropriate activation signal. This signal can be provided by a clonally-distributed, specific antigen receptor (Ti-CD3), or through certain broadly distributed cell membrane structures. Molecular biological and in vitro cell culture techniques have been used to investigate the expression of the genes encoding these activation structures, and to study the relationship between structure and function.

Analysis of cells possessing competing functional α and β chains unexpectedly revealed striking asymmetries in the efficiency of expression of certain chain combinations. The variation between two given combinations could be as great as 20x. Much of this difference can be attributed to the germline Vβ segment, implying that this region interacts with the α chain in a structurally constrained manner. This observation suggests that the T cell repertoire may be limited in part by the failure of certain αβ combinations to yield enough surface receptor for function.

Experiments with matched cell pairs that either expressed or failed to express the Ti-CD3 complex due to defects in synthesis of one or the other of the αβ chains demonstrated that signalling through some surface molecules (e.g. CD2) after cross-linking with monoclonal antibodies, did not require CD3 expression. However, such signalling was enhanced by the simultaneous expression of CD3 by the target cell. These results have important implications for the pathways of transmembrane signalling involving the TCR and other surface structures.

Additional experiments are in progress to use our gene transfer and expression techniques for TCR to evaluate the significance of the simultaneous expression by a single cloned T cell of two in-frame β gene transcripts and β proteins, and to study the importance of junctional diversity in the specificity of γδ TCR. Attempts are also being made to construct transgenic mice expressing desired TCR chains in the majority of T cells, to help study T cell ontogeny and the peripheral mechanisms of T cell tolerance.

These studies will enhance our knowledge of which cell membrane molecules are involved in triggering T cells to exert regulatory and effector function, and our understanding of the structural basis for specific T cell responses to antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00425-05 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Chused	Senior Investigator	LI, NIAID
Others:	Y. A. Ishida	Visiting Fellow	LI, NIAID
	E. M. Brown	Biologist	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive "feed-forward" and "feed-back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00427-05 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen-Specific and Antigen-nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Visiting Scientist	LI, NIAID
Others:	R. Taffs	Staff Fellow	LI, NIAID
	A. Filippini	Visiting Fellow	LI, NIAID
	G. Trenn	Visiting Associate	LI, NIAID
	T. Sugiyama	Visiting Fellow	LI, NIAID
	T. Agui	Visiting Associate	LI, NIAID
	W. Humphrey	Research Technician	LI, NIAID
	M. Piper	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To identify proteins (enzymes) involved in T-cell receptor (TCR)-triggered mechano-biochemical activities of cytotoxic T-lymphocytes (CTL), we studied the biochemical changes in cloned CTL after occupation of functionally important surface proteins by antigens or mAb. The ultimate goal of these studies is to understand molecular mechanisms of cytotoxicity and to design specific drugs/peptides that will modulate the intensity of effector functions of lymphocytes.

Biochemistry and Immunopharmacology. We implicated the calmodulin-dependent phosphatase, calcineurin, in activation pathway of CTL and we used the same methodological approaches to clarify the question of calmodulin being the molecular target for cyclosporin A (CsA). We found that CsA markedly inhibits target cell- and anti-TCR mAb-induced granule exocytosis in CTL and IgE-receptor mediated exocytosis in basophilic cells. Thus, CsA is implicated in affecting degranulation-mediated processes in vivo. A differential screening procedure for the search for new, CsA-like proteins, is suggested. Since we considered the cAMP-dependent protein kinase (PK-A) as an integral part of an "OFF" signal, we suggested the use of an antagonist of cAMP (Rp-cAMPS) as an inhibitor of PK-A and, potentially, as a immunoenhancer.

Mechanisms of Cytotoxicity. We attempted to isolate and to identify polypeptides that are secreted by TCR-activated CTL. It is found in studies of ^{35}S-methionine labelled CTL that polypeptides with apparent M.W. Forty-two, 36 and 31 KDa are secreted constitutively, while 23.5, 18.5 and 17 KDa are secreted only after TCR crosslinking. None of these polypeptides was released in the absence of Ca^{++}. Thus, they can not be a part of a "lytic hit" by CTL. A novel mechanism of cytotoxicity is proposed. We suggested the consideration of the extracellular ATP as a lytic intermediate of a CTL-mediated cytotoxicity in situations when poreforin cannot account for target cell lysis. Experimental data support this, since: i) ATP can kill target cells, while CTL are protected by high levels of ecto-ATPase activity, ii) CTL secrete ATP, iii) ATP kills target cells in the absence of Ca^{++}.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00493-03 LI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
B Cell Stimulatory Factor-1 (BSF-1)/Interleukin-4(IL-4)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Paul	Chief	LI, NIAID
Others:	J. Ohara	Visiting Scientist	LI, NIAID
	G. LeGros	Visiting Fellow	LI, NIAID
	S. Z. Ben-Sasson	Visiting Scientist	LI, NIAID
	U. Atasoy	NRSA Fellow	LI, NIAID
	A. Keegan	NRC Fellow	LI, NIAID
	D. Conrad	Guest Researcher	LI, NIAID
	M. Plaut	Guest Researcher	LI, NIAID

COOPERATING UNITS (if any)
NCI (J. Pierce, J. Hanley-Hyde, R. Nordan); Uniformed Services Univ. Health Sciences (F. Finkelman, I. Katona); Dept of Agriculture (J. Urban); NY Univ. School of Medicine (P. D'Eustachio)

LAB/BRANCH
Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.85	4.0	1.85

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interleukin-4 (IL-4) is a lymphokine with potent effects on a variety of hematopoeitic and some non-hematopoietic cells. It has recently been shown that the IL-4 gene is found on murine chromosome 5 and is linked to the IL-3 gene. Others have shown, that iL-4 and IL-5 are closely linked, as are IL-3 and GM-CSF. This suggests that IL-4, IL-5, GM-CSF and IL-3 constitute a related family of lymphokines. Non-transformed IL-3-dependent mast cell lines produce this set of lymphokines, but not IL-2, interferon gamma (IFNγ) or lymphotoxin, in response to cross-linkage of high affinity Fcε receptors or to treatment with calcium ionophores. A population of spleen cells exists, lacking both B and T cell markers, that releases IL-4 and IL-3, but not IL-2 or IFNγ, in response to polymerized IgE or IgGs. In non-immunized mice, the capacity of non-B, non-T cells to produce IL-4 is dependent on a differentiation step mediated by IL-3.

Among the most striking activities of IL-4 is its in vitro control B cell switching to the expression of IgG1 and IgE and its in vivo regulation of IgE responses. Treatment with anti-IL-4 antibodies not only blocks primary IgE responses, it is also a striking and selective inhibitor of secondary IgE responses to both nematode reinfection and to boosting with hapten-protein conjugates. Not only is IL-4 required for IgE responses, forms of immunization associated with increased IgE production are also marked by a major increase (40-50 fold) in T cell IL-4 production in response to anti-CD3 antibody and in non-B, non-T cell IL-4 production in response to polymerized IgE or IgG2a. These results indicate that regulation of production of IL-4 and other members of the "IL-4 lymphokine set" by T cells and possibly by non-B, Non-T cells are critical regulators of mIgE production and of "allergic inflammation".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00520-02 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. N. Germain	Section Head	LI, NIAID

COOPERATING UNITS *(if any)*

LVD, NIAID (B. Moss); MC, NCI (H. Takahashi, J. Berzofsky)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop effective therapeutic and vaccine strategies to combat infection with HIV-1, the cause of AIDS, it is essential to understand the cell-mediated immune response to this infectious agent. Towards this end, model systems for immunization with HIV proteins are being devised and used to test the ability of these proteins to stimulate various subsets of T lymphocytes.

Initial experiments have utilized a mouse model. Animals were immunized with recombinant vaccinia virus containing an expressible form of the env gene of the IIIB strain of HIV-1, encoding the gp160 molecule. Lymphoid cells from such immunized mice restimulated in vivo were found to contain cytotoxic T lymphocytes (CTL) with specificity for the gp160 protein in the context of $H\text{-}2D^d$. No such responses were observed with cells from $H\text{-}2^k$ or $H\text{-}2^b$ mice.

Synthetic peptides corresponding to segments of the gp160 molecule were used to map the sites being recognized by these CTL. Only a single major recognition site was identified. This site was in an area of extensive sequence variation among isolates of HIV-1. The effects of this sequence variation on immunogenecity of this sequence was examined. CTL were raised by vaccination with recombinant vaccinia virus expressing the gp160 MN sequence, followed by in vitro stimulation with a peptide corresponding to the identified IIIB site but possessing the corresponding MN residues. Such CTL could lyse vaccinia gp160 MN but not vaccinia gp160 IIIB targets, and MN peptide but not IIIB peptide-coated targets. Alteration in a single amino acid at position 325 of the gp160 molecule completely switched the specificity of these peptides for the two CTL populations, indicating that the vial isolates differed in the epitope of this critical CTL determinant.

These data indicate that in HIV proteins the immunodominant regions may be subject to strong selection in vivo. In addition to the epitope variation noted above, a possible example of a change in the MHC binding site of the gp160 CTL determined has also been observed. These findings have implications for the mode of viral escape from immune effectors and the possible basis for progression from HIV seropositivity to AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00545-01 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. N. Germain	Section Head	LI, NIAID
Others:	G. Otten	Special Volunteer	LI, NIAID
	J. Sherman	Medical Staff Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.75	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The manner in which antigen is acquired, processed, and presented to T lymphocytes is critical to the immunity developed by the organism. It has become clear that αβ T cells are limited to recognition of derivatives of protein antigens presented on cell surfaces in association with MHC products. The manner in which intact proteins are taken up by or synthesized within cells, and the subsequent alteration in their structure that makes them able to bind in an effective manner to class I and class II MHC molecules is an area of active investigation.

Several years ago, we proposed that there were two fundamentally distinct routes followed by antigens: one involved molecules acquired by the cell into endosomes and the other the biosynthesis or direct introduction of protein into the cytoplasm. Proteins entering the endocytic pathway were considered destined for class II MHC presentation, whereas those found in the cytoplasm became associated with class I MHC molecules. Our own unpublished studies and several appearing recently in the literature support this general model. Thus, intact ovalbumin taken up into endosomes is presented exclusively by the class II pathway, whereas ovalbumin made by transfected cells or introduced into the cytoplasm by osmotic lysis becomes available to the class I pathway.

These general rules suggest that the process of self-tolerance to tissue-specific antigens must differ for class I-restricted and class II-restricted T cells, with the former leaving the thymic environment still able to respond to self. Preliminary studies confirm this prediction, and suggest that control of self-reactivity of such class I-restricted cells occurs peripherally by a clonal anergy mechanism. This mechanism may be related to, although distinct in detail from, our recent observation of unresponsiveness induced in $CD8^+$ IL-2 producing cells by exposure to ligand in the absence of an undefined "co-stimulator" possessed by traditional antigen presenting cells.

LIP

LABORATORY OF IMMUNOPATHOLOGY
1989 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1988 to September 30, 1989

Herbert C. Morse III, M.D.
Chief, Laboratory of Immunopathology

The major programs in the Laboratory of Immunopathology are centered on developing an understanding of the mechanisms of virus-induced neoplasia with emphasis on murine leukemia viruses (MuLV) and papovaviruses. Over the last four years, increasing importance has been placed on studies of a MuLV-induced immunodeficiency syndrome of mice, termed MAIDS, with many similarities to AIDS in man. Other studies of substance include i) analyses of patterns of hematopoietic differentiation and function with a prominent focus on B cells that express CD5+; ii) investigations of oncogenes that transform hematopoietic cells including a new oncogene discovered in the laboratory; and iii) evaluations of mutant genes associated with development of autoimmune disease.

Considerable progress has been made in defining the host-virus interactions responsible for the development of immunodeficiency in the MAIDS model system, including identification of a defective virus as the disease-causing agent and characterization of host genes influencing the development of disease.

A defective virus is required for induction of MAIDS. Stocks of virus, termed LP-BM5 MuLV, that cause MAIDS were previously shown to include replication competent ecotropic and mink cell focus-inducing (MCF) MuLV. Mice inoculated with a series of biologically cloned viruses of either class failed to develop disease. Molecular studies of the virus mixture revealed the presence of a defective 4.8 kb virus, lacking most of env and pol, that was required for disease. (Chattopadhyay, Morse, Makino, Ruscetti, Hartley).

MHC genes are involved in resistance to mouse acquired immunodeficiency disease (MAIDS). In $\underline{Fv\text{-}1}^b$ mouse strains, genetically permissive to infection by the B-tropic helper viruses in LP-BM5 MuLV stocks, genes in or linked to H-2 have a marked effect on development of MAIDS, with resistance being associated with reduced expression of ecotropic and MCF viruses. Mice bearing the $\underline{H\text{-}2}^b$ haplotype develop severe disease of short latency while $\underline{H\text{-}2}^d$ strains are strongly resistant, showing mild or no disease or developing MAIDS only after extended latent periods. (Makino, Hartley, Fredrickson, Morse).

Resistance to MAIDS is complex. The major site of MHC association with resistance is at H-2D but other regions within H-2 may interact with D end genes to influence the course of disease and replication of virus. Background genes are also seen to affect development of MAIDS. Resistance is recessive in crosses between susceptible and resistant strains. (Makino, Hartley, Fredrickson, Morse).

MAIDS can occur in mice of Fv-$1^{n/n}$ and Fv-$1^{n/b}$ genotype. The unexpected development of disease in certain mice with Fv-1-based restriction of replication of B-tropic LP-BM5 helper MuLVs appears to be based on ability of these mice to generate helper viruses for which they are permissive. These viruses may derive from induction of endogenous N-tropic ecotropic MuLV sequences, or by mutation to or selection of NB-tropic variants. (Hartley, Fredrickson, Morse).

Mechanism of T-cell lymphoma induction by Moloney MuLV is related to transcriptional activity in specific target cells. Mouse pathogenicity tests of virus constructs which include sequences from a set of Moloney MuLVs with point mutations introduced into binding sites on the viral enhancer have shown that mutations decreasing transcriptional activity in T cells also result in increased latency for lymphoma induction. (Speck, Hopkins, Fredrickson, Hartley).

B cells are required for development of T cell abnormalities in MAIDS. To determine if abnormalities of antigen-presenting cells contributed to T cell abnormalities in this syndrome, mice were depleted of B cells from birth with anti-IgM antibodies and infected with LP-BM5 MuLV. T cells from treated animals were found to be completely normal in their ability to respond to mitogenic and antigenic stimuli. (Cerny, Hugin, Makino, Hayakawa, Hardy, Holmes, Zinkernagle, Morse).

Expansion of a subset of $CD4^+$ T cells in MAIDS. In normal mice, $CD4^+$ cells can be divided into four populations, based on expression of two cell surface markers, SM3G11 and SM6C10. The subset of cells expressing neither marker makes up 10% of cells in normals but, remarkably more than 85% of cells in mice with advanced MAIDS. Many of these cells are also Thy-1^-, a phenotype expressed by only 10% of normal $CD4^+$ cells. (Holmes, Hardy, Hayakawa, Morse).

In studies of oncogenes, we analyzed several characteristics of cbl, raf and myc.

Mapping of cbl in mouse and man. The v-cbl oncogene induces pre-B cell lymphomas in vivo and transforms fibroblasts in vitro. Cellular sequences related to the viral gene were mapped in the mouse to chromosome 6 and chromosome 9. The chromosome 6 locus appears to be a processed gene while that on 9 is a complex gene. In man, CBL was mapped to chromosome 11q23 in a region involved with translocations in a variety of leukemias and lymphomas.

(Regnier, Langdon, Shapiro, Jenkins, Copeland, Kingsley, Savage, Kersey, Croce, Morse).

In vitro induction of early T cell lineage lymphomas with raf and myc Infection of 14 day fetal thymus organ cultures with a retrovirus containing v-raf and v-myc resulted in the development of early T cell lineage tumors expressing T cell receptors, but lacking expression of CD4 and CD8. These lines should prove useful in dissecting early events in T cell differentiation and function. (Principato, Kruisbeek, Rapp, Morse).

Two distinct autosomal recessive mutations of the mouse, lpr and gld, are associated with autoimmunity and expansion of an unusual subset of T lymphocyte that fail to respond to mitogenic or antigenic stimuli. We have extended our studies of the characteristics of these cells as well as evaluating features of $CD5^+$ B cells that are suggested to contribute to autoantibody production.

The expanded populations of $CD4^-$ $CD8^-$ T cells of lpr and gld mice are $CD2^-$. The abnormal T cell characteristic of these mutations were analyzed for expression of CD2, a cell surface molecule involved in adhesion and activation of T cells through interactions with LFA-3. Cells from both mutants were found to be $CD2^-$ at both the protein and mRNA levels. Minor populations of thymic and splenic T cells with similar phenotypes were identified. (Shirai, Abe, Yagita, Okumura, Davidson, Morse).

Deficiency of $CD5^+$ B cells in RIIIS/J mice. Mice of this strain were found to express low levels of antibodies to autoantigens while responding normally to conventional antigens. This phenotype was associated with low levels of $CD5^+$ B cells with frequencies of other B cell subsets being near normal. These observations reinforce the relationship between autoantibody production and $CD5^+$ B cells. (Holmes, Hiernaux, Baker).

Studies of DNA tumor viruses have elucidated the complex interactions of cells transformed by these viruses with the immune system as well as suggesting novel mechanisms in tumor progression.

NK cell competence determines the tumorigenicity of DNA virus transformed cells in athymic rodents. Adenovirus 2 (Ad2) transformed rat and hamster cells are susceptible to lysis by NK cells from the host of origin and are nontumorigenic in these species. Studies of nude rats showed that their NK cells lysed Ad2 cells from both species and that they were resistant to growth of these lines. In contrast, NK cells from nude mice were incapable of lysing the cells and the mice were susceptible to tumor formation. These results point out important differences in the nonspecific host cellular responses to Ad-transformed cell lines in athymic animals. (Cook, Lewis).

Relationship of Adenovirus gene expression to transformation of mouse embryo cells. Mouse embryo cells transformed by Ad5 were found to vary considerably in the content and expression of the virus genome. The spectrum ranged from integration and expression of the E1A and E1B transcriptional units, to integration without expression of these genes, to initial integration followed by sequential shutdown of translation and transcription. These results indicate that continued expression of AD5 genes is not required for maintenance of the transformed state and may have important implications for the understanding of DNA virus tumorigenicity. (Carbone, Chattopadhyay, Lewis).

Production of an antiproliferative factor, mitogenic inhibitor (MI), does not correlate with tumorigenicity of SV40 transformed hamster cells. MI, produced by hamster cells transformed in vitro by SV40, has a strong antiproliferative effect on immune effector cells. The possibility that production of MI was an important determinant of the tumorigenicity of SV40 transformed lines was excluded by the observations that 6 of 11 tumors induced *in vivo* by the virus did not produce MI. However production of MI did correlate with the morpholology and growth characteristics of cells *in vitro*. (Carbone, Dixon, Lewis).

Administrative Changes. Dr. Michele Carbone transferred to NICHHD after completing his tenure as a Fogarty Visiting Fellow. Drs. Silvia Soddu and Daniel Regnier moved into Fogarty Visiting Fellow positions and Dr. Ambros Hugin joined the Laboratory as a Fogarty Visiting Associate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00013-26 LIP

PERIOD COVERED

October 1, 1987 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	A. M. Lewis, Jr.	Hd, Viral Pathogenesis Section	LIP, NIAID
Others:	M. Carbone	Visiting Fellow	LIP, NIAID
	S. Soddu	Visiting Fellow	LIP, NIAID
	J. A. Sogn	Senior Investigator	LIG, NIAID
	A. S. Levine	Scientific Director	OSD, NICHD
	C. T. Patch	Senior Investigator	OSD, NICHD
	K. Dixon	Senior Investigator	OSD, NICHD

COOPERATING UNITS *(if any)*

National Jewish Center for Immunology and Respiratory Medicine, Denver, CO (J. L. Cook).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The E1A gene of nononcogenic Ad2 and Ad5 induces susceptibility to NK cell and macrophage mediated lysis in transformed rodent cells. The expression of high levels of susceptibility correlates with transformed cell tumor inducing capacity. Adaptation of Ad2 transformed cells to produce tumors in syngeneic adult hamsters by serial passage in newborns, results in cells that are resistant to NK cell and macrophage killing and whose E1A gene function is disrupted. We have also found that Ad12 transformed hamster and mouse cells that have low levels of class I MHC antigens on their surfaces are generally nontumorigenic in allogeneic hamsters and mice and are highly immunogenic in bioassays for CTL responsive Ad12 transplantation antigens. Our Ad12 transformed BALB/c mouse cells produced tumors in BALB/c and DBA-2 mice but are rejected as allografts by H-2 divergent mice inspite of their low levels of class I MHC antigen expression. Cells derived from Ad12 transformed mouse cell induced tumors express the same tumorigenic phenotype. Ad5 transformed BALB/c mouse cells that contain Ad5 E1A genes may or may not express Ad5 E1 RNA or proteins. Some cells that initially contain and express Ad5 E1 functions lose this capacity during tissue culture passage. Some cells that are morphologically similar to the others contain no Ad5 DNA. Cells that fail to express or lose their capacity to express Ad5 E1A functions are more tumorigenic than those cells that express Ad5 E1 functions. This model may help explain how certain DNA viruses that are putatively tumorigenic in humans and animals produce tumor cells that may or may not contain an expressed viral functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00135-15 LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	J. W. Hartley	Senior Investigator	LIP, NIAID
	S. K. Ruscetti	Investigator	LG, NCI
	D. Regnier	Visiting Fellow	LIP, NIAID
	M. Shapiro	Investigator	LIP, NIAID
	N. Copeland	Chief	MGL, NCI
	N. Jenkins	Senior Investigator	MGL, NCI
	D. Kingsley	Investigator	MGL, NCI

COOPERATING UNITS *(if any)*

Walter and Eliza Hall Institute, Melbourne, Australia (W. Y. Langdon, S. P. Klinken).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Altered structure and/or expression of cellular proto-oncogenes is associated with the development of neoplasms in vertebrates. We have isolated, cloned and sequenced a new oncogene, termed v-cbl, that was captured by a retrovirus that induces pre-B cell lymphomas in vivo. Cellular sequences related to v-cbl have been chromosomally mapped in mouse and man. The mouse genome contains two loci with homology to v-cbl. One locus, designated Cbl-1, is located on chromosome 6 and has the structure of a processed gene. The second locus, termed Cbl-2, is located on chromosome 9 and has the structure of a complex gene. Mapping studies in man have localized a single v-cbl-related sequence, termed CBL, to chromosome 11q23, a region frequently involved with translocations in human leukemias and lymphomas. In both mouse and man, cellular homologs of v-cbl are expressed as 11.0 kb transcripts in most tissues, but with smaller transcripts predominating in testis. Antibodies made to v-cbl protein expressed in bacteria recognize a 135 kD protein that is tightly associated with the nucleus. The nuclear localization of the cbl product and its homology with the yeast gene GCN4 suggest that it may function as a DNA binding protein that activates transcription. Genes with these functions have been suggested to be important for controlling the entry of cells into cycle. Further studies will be directed at determining the structure of cbl genes in normal cells and neoplasms of the B cell and myeloid lineages.

PHS 6040 (Rev 1/84)

GPO 914-918

Other Investigators:

C. A. Kozak	Investigator	LMM, NIAID
U. R. Rapp	Senior Investigator	LVC, NCI
M. A. Principato	Investigator	LIP, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00138-15 LIP

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Viruses and Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	J. W. Hartley	Senior Investigator	LIP, NIAID
	K. L. Holmes	Investigator	BRB, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	R. M. L. Buller	Senior Staff Fellow	LVD, NIAID
	A. Singer	Chief	LEI, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunopathology

SECTION
Virology and Cellular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Inactive this year.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00205-09 LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	W. F. Davidson	Visiting Scientist	LG, NCI
	C. Calkins	Guest Researcher	LIP, NIAID

COOPERATING UNITS *(if any)*

Juntendo University, Tokyo, Japan (T. Shirai, K. Okomura).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic and environmental factors interact in the development of autoimmune disease in mouse and man. In the mouse, two autosomal recessive mutations, lpr, and gld, contribute to syndromes characterized by production of lymphoproliferation and autoantibodies. To evaluate mechanisms by which these mutations result in autoimmunity, we have studied the characteristics of the expanded populations of Thy-1^+, $CD4^-$, $CD8^-$, TCR αB^+ cells present in the lymph nodes and spleens of the mutant mice. We found that these cells do not express a cell surface molecule, CD2, present on almost all normal T cells that facilitates antigen recognition. Failure to express CD2 protein was shown to be controlled at the level of transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00284-08
LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	J. W. Hartley	Hd, Viral Oncology Section	LIP, NIAID
Others:	H. C. Morse III	Chief	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	K. L. Holmes	Senior Investigator	BRB, NIAID
	S. K. Chattopadhyay	Expert	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	R. Repaske	Senior Investigator	LMM, NIAID
	S. Ruscetti	Investigator	NCI

COOPERATING UNITS *(if any)*

Massachusetts Institute of Technology, Cambridge, MA (N. Hopkins, N. Speck); Univ. of Massachusetts, Worcester, MA (C. Holland); American Red Cross, Bethesda, MD (G. Jay).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	1.1	2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the isolation and biological and molecular characterization of C-type murine leukemia viruses (MuLVs), with major current emphasis on the molecular dissection of the viral genome sequences specifying induction of erythroleukemia or T-cell lymphoma by Friend MuLV and Moloney MuLV, respectively; and on the identification and analysis of the specific component of the LP-BM5 virus mixture responsible for induction of the severe immunosuppression and lymphoproliferation characterizing mouse acquired efficiency disease (MAIDS). Utilizing reciprocal recombinant viruses constructed between Friend and Moloney MuLVs we have shown that disease specificity is determined by sequences within the U3 region of the viral LTR, which contains viral transcriptional elements. Although the effective sequences have been further narrowed to those encoding the enhancer region, studies using additional recombinants and site-directed mutants have shown that the determinants cannot be localized to a single site. Rather, disease specificity is determined by several regions within the enhancer, genomic sequences outside the LTR can make some contribution, and certain combinations of enhancer sequences can affect disease latent period. From molecular and biological studies of LP-BM5 MuLV infection the direct etiologic agent of MAIDS has been determined to be a 4.8 kb genome with deletions and substitutions in *gag*, *pol*, and *env* coding regions. This defective genome requires coinfection with competent ecotropic or MCF MuLV in order to establish *in vitro* and *in vivo* infection. Molecularly cloned full length and defective genomes have been obtained which will permit definition and manipulation of virus populations in studies to determine critical biologic properties and the interactions of LP-BM5 MuLVs in inducing disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00286-08 LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	J. W. Hartley	Hd, Viral Oncology Section	LIP, NIAID
Others:	M. Makino	Visiting Fellow	LIP, NIAID
	H. C. Morse III	Laboratory Chief	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID

COOPERATING UNITS *(if any)*

Tufts University School of Medicine, Boston, MA (J. Stoye).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	1.75	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of host genes are known to influence or regulate infection of mice by murine leukemia viruses (MuLVs) and to influence development of disease in successfully infected animals. Major emphasis has been on the study of host genes involved in determining sensitivity or resistance to retrovirus-related mouse acquired immunodeficiency diseae (MAIDS), a syndrome induced by LP-BM5 MuLV, which comprises a replication defective genome and helper ecotropic or MCF MuLV. Restriction of B-tropic helper MuLV infection and spread is strongly affected by the mouse Fv-1 locus; thus the most sensitive strains are $\underline{Fv\text{-}1}^b$ and strains of $\underline{Fv\text{-}1}^n$ genotype are highly or partially resistant. However, some $\underline{Fv\text{-}1}^b$ strains may be highly resistant - C57BL and I/St are very susceptible but A and BALB/c are resistant. The influence of H-2-linked loci has been explored using recombinant inbred mice and H-2 congenic, mutant, and recombinant mouse strains. $\underline{H\text{-}2}^b$ haplotype mice were found to be regularly sensitive while $\underline{H\text{-}2}^d$ mice were resistant, resistance being associated with reduced expression of ecotropic and MCF MuLVs. Positive and negative effects of background genes were observed. Although influences of other genes within H-2 were evident in certain combinations a crucial role was delineated for $\underline{H\text{-}2D}^d$ region genes in resistance. Surprisingly certain $\underline{Fv\text{-}1}^n$ strains, notably P, BDP, and AKR show moderate sensitivity to MAIDS and (C57BL/6 x CBA/N)F1 mice ($\underline{Fv\text{-}1}^{n/b}$ and thus dually restrictive) are of relatively high susceptibility. The results of virus recovery tests suggest that apparently anomalous sensitivities based on predicted Fv-1 restriction, may reflect induction of endogenous N-tropic MuLV or mutation of B-tropic virus to NB-tropism thus providing a helper virus for which the host is permissive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00465-04
LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retrovirus-induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	J. W. Hartley	Senior Investigator	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	G. Shearer	Investigator	LEI, NCI
	J. R. Wunderlich	Investigator	LEI, NCI
	K. L. Holmes	Investigator	BRB, NIAID
	S. K. Chattopadhyay	Expert	LIP, NIAID
	D. M. Klinman	Investigator	IDB, NINCDS

COOPERATING UNITS *(if any)*

Fox Chase Cancer Center, Philadelphia, PA (K. Hayakawa, R. Hardy); Johns Hopkins Medical School, Baltimore, MD (P. Pitha), Veterans Administration Medical Center, Baltimore, MD (R. A. Yetterr).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mice infected with retroviruses, termed LP-BM5 murine leukemia viruses (MuLV), develop a syndrome characterized by profound immunodeficiency and lymphoproliferation that has many similarities to AIDS in man. We have investigated the host-virus interactions that result in impairment of immune function. B cell abnormalities in this syndrome include early activation and differentiation to immunoglobulin secretion and later development of lymphomas. Studies of early events showed that B cell activation was a true polyclonal expansion of the normally expressed repertoire. At later times after infection, the repertoire diverged in association with the development of oligoclonal populations of cells. Induction of B cell abnormalities was shown to depend on the presence of activated T cells of CD4$^+$ phenotype. To understand how these cells were activated, mice were depleted of B cells. This manipulation prevented the development of T cell abnormalities, indicating a complex circuitry of interactions between these sets of lyumphocytes. A role for various lymphokines in these processes was investigated by examining mRNA transcripts for IL-1, -2, -3, -4, and -6 in spleen and lymph nodes. Infection was found to stimulate expression of message for IL-1 but not the other lymphokines. Analyses of host determinants of resistance to disease demonstrated a prominent role for Fv-1, but showed that this resistance could be overcome by induction or mutation to provide helper viruses for which the host is permissive. Additional major influences on disease and virus expression were effected by genes of the major histocompatibility complex.

Project Number Z01 AI 00465-04 LIP

Other Investigators:

T. N. Fredrickson	Research Microbiologist	LIP, NIAID
A. Cerny	Guest Researcher	LIP, NIAID
A. Hugin	Visiting Fellow	LVD, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00484-03 LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	K.L. Holmes	Hd, Flow Cytometry Section	BRB, NIAID
Others:	H.C. Morse, III	Chief	LIP, NIAID
	D. Klinman	Investigator	IDB,NINCDS
	W.F. Davidson	Visiting Scientist	LG,NCI
	Hiernaux, J.R.	Visiting Fellow	LI, NIAID
	Baker, P.J.	Senior Investigator	LI, NIAID
	Makino, M.	Visiting Fellow	LIP,NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously suggested that the B cell and macrophage lineages may share a common progenitor cell. Evidence has been accrued in support of this hypothesis. We have shown that the precursor for B and T cells expresses Ly-40(CD11b), an antigen present on mature macrophages and granulocytes. A monoclonal antibody (MAb), LIP-6, was developed that recognizes an antigen expressed by B cells and macrophages. We have recently found that the Ly-1(CD5) antigen is common to macrophages and peritoneal B cells. The LIP-6 antigen is also expressed by pre-B cells and may be found on early B cell precursors.

Studies of the functional differences in Ly-1(CD5)$^+$ B cells have shown that the anti-bromelain and anti-phosphorylcholine responses are higher in the Ly-1(CD5) lineage (as predicted) but that the frequency of cells that spontaneously secrete antibody is also higher in peritoneal Ly-1(CD5)$^+$ B cells but not splenic Ly-1(CD5)$^+$ B cells.

In LP-BM5 infected mice, a novel T helper cell subset that lacks Thy-1 expression was found to undergo expansion with progression of disease. This subset is detectable at low frequency in normal mice and includes the helper cell subset known as Th1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00544-01
LIP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis of Pathogenesis of Murine Leukemia Virus Infections.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	S. K. Chattopadhyay	Expert	LIP, NIAID
Others:	H. C. Morse III	Chief	LIP, NIAID
	J. W. Hartley	Hd, Viral Oncology Section	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	S. K. Ruscetti	Investigator	LG, NCI

COOPERATING UNITS *(if any)*

Georgetown University, Washington, DC (B. Baroudy); University of Massachusetts Medical Center, Worcester, MA (C. Holland); Memorial Sloan-Kettering Cancer Center, NY (P. V. O'Donnell).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general purpose of this project is the molecular characterization of various pathogenic murine leukemia viruses (MuLVs) with the ultimate goal of determining the molecular basis of pathogenicity. Three systems have been used: (a) helper-independent highly pathogenic (T-cell lymphoma) MCF virus NS6(186); (b) helper-dependent thymotropic nonleukemogenic AKR MCF 30-2; and (c) a mixture of MuLVs, termed LP-BM5 MuLV, which upon infection in susceptible mice develop a syndrome characterized by lymphoproliferation and immunodeficiency. Detailed restriction endonuclease maps of these viruses were derived from Hirt supernatant DNA and biologically active molecular clones were derived. It has been shown that both NS6(186)MCF and AKR 30-2 MCF are recombinants of host cellular MCF related sequences and the parental ecotropic viruses Cas-Br-M and Akv respectively. The long terminal repeat sequences and the envelope gene of these viruses have been sequenced. Analyses of these sequences established that the number of copies of the enhancer element in the U3 region of LTRs greatly influence the thymotropic and oncogenic phenotype of the virus. In efforts to determine which of the components of LP-BM5 mixture, a tissue culture stock known to contain both B-tropic ecotropic and MCF MuLVs, several biologically cloned ecotropic and MCF isolates failed to induce disease, alone or in combination. Biological manipulation and biochemical analyses enabled us to determine that disease induction correlated with the presence of a 4.8 kb defective genome, which cross reacts with MCF env sequences and is dependent upon ecotropic helper virus for replication. Both the ecotropic helper and the defective genomes are now molecularly cloned.

LIR

LABORATORY OF IMMUNOREGULATION
1989 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1988 through September 30, 1989

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation

Immunopathogenic Mechanisms of HIV Infection

Over the past several years the LIR has been involved in studying the mechanisms of immunopathogenesis of human immunodeficiency virus (HIV) infection. Most recently we have been intensively investigating the cellular and molecular mechanism whereby a latent or low level chronic HIV infection is converted to an active productive infection leading to lymphocyte depletion and immune suppression. We had previously established a model system of chronically HIV-infected, cloned T cell (ACH2) and promonocytic (U1) lines which constitutively expressed little or no HIV, but could be induced to express HIV in up to 100% of cells upon stimulation with phorbol myristate acetate (PMA). Having previously demonstrated that crude cytokine enriched supernatants could induce expression of HIV in these cell lines, over the past year we demonstrated that tumor necrosis factor-alpha (TNF-α) could induce HIV expression in ACH2 cells, and interleukin-6 (IL-6) as well as granulocyte macrophage-colony stimulating factor (GM-CSF) could induce HIV expression alone or in synergistic combination with TNF in U1 cells. These findings are of particular relevance since these cytokines are of physiologic importance and TNF levels are elevated in the plasma of HIV-infected individuals to concentrations that are capable of inducing HIV in vitro.

At the molecular level, we demonstrated that TNF induces HIV expression in ACH2 cells through the induction of transcription activating factors that bind to the NF-kB sequences in the HIV long terminal repeat (LTR). Furthermore, we clearly demonstrated that TNF can function in an autocrine manner in the induction of HIV expression in our chronically infected cell lines. Briefly, endogenous TNF could be produced and secreted in ACH2 and U1 cells in response to PMA stimulation which induces HIV expression. Also, exogenous TNF synergizes with PMA in the induction of TNF secretion. Furthermore, PMA induction of ACH2 and U1 results in the upregulation of TNF receptor expression. Finally, antibody to TNF could block the PMA-induction of HIV expression.

In other studies of potential co-factors in HIV expression, we demonstrated that ultraviolet (UV) light as well as heat shock can induce HIV. Of note is the fact that transient increases in temperature to 40°C can synergize with TNF and IL-6 in the induction of HIV. Also, antigens of viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV) and HIV were shown to induce monocytes to secrete factors, one of which is TNF which can induce HIV expression.

We have demonstrated that monocyte tropism is likely an in vitro phenomenon since virus which could only be isolated onto monocytes was shown by polymerase chain reaction (PCR) to be contained in $CD4^+$ cells. In fact, we have demonstrated that the primary reservoir of HIV in the peripheral blood is the $CD4^+$ T cell which maintains its expression of CD4. Also, contrary to studies which demonstrated a frequency of HIV expression of 1:10,000 to 1:100,000 peripheral blood cells in acquired immunodeficiency syndrome (AIDS) patients, we demonstrated by PCR that HIV provirus is contained in $CD4^+$ cells of AIDS patients at a frequency of greater than 1:100.

We identified and were able to propagate in vitro and characterize a $CD3^+/CD4^+/CD8^+$ cell line from a patient with HIV-1 infection. This cell line was found to express a unique IL-2 receptor and to constitutively express HIV-1 proteins. The level of HIV-1 production could be induced with a variety of stimuli, providing a model for in vivo regulation of HIV-1 replication.

The effects of HIV-1 infection on T cell activation were studied utilizing in vitro infection of peripheral blood $CD4^+$ T cells and transfection of T cell lines. Infection with HIV-1 was found to select for the outgrowth of gamma-delta T cell lines. These gamma-delta T cell lines as well as gamma-delta T cell lines derived from patients with HIV-1 infection are being characterized at the level of their T cell antigen receptor. In addition, we have established and have begun the characterization of a series of T cell lines transfected with the nef gene of HIV-1. (Fauci, Poli, Schnittman, Massari, Stanley, Duh, Lane, Bressler, Dayton, Fox, LIR/NIAID; Rabson, LMM/NIAID; Clouse, Georgetown).

The Immune Response to HIV Infection

The cytotoxic T cell response to HIV has been investigated using freshly separated and cloned peripheral blood T cells derived from HIV-seropositive individuals as effector cells and EBV-transformed B cells infected with recombinant vaccinia viruses expressing different HIV-proteins as target cells. We have demonstrated the presence of HIV-specific cytotoxic T lymphocytes (CTL) to env, gag, pol, and nef proteins in unfractionated and cloned cell populations. Using recombinant vaccinia viruses expressing truncated forms of the envelope protein, we have delineated several regions within the gp120 and 41 envelope proteins that induce HIV-specific CTL. Using target cells pulsed with peptides synthesized from gag and nef sequences, we have begun to define the specific amino acid sequences which stimulate CTL responses. We have demonstrated that two HIV-seropositive individuals recognize the same nef peptide in the context of the major histocompatability complex (MHC) A3 allele. We will continue in this effort to generate a catalogue of epitopes stimulating HIV-specific CTL. This information will be critical in designing an effective vaccine for HIV.

In our investigations of the role of $CD8^+$ T cells in the containment of HIV infection, we discovered that AIDS patients had in their peripheral blood an increased percentage of activated $CD8^+$ cells which expressed DR antigen but had the unusual phenotype of being CD25 (IL-2 receptor) negative. These cells also expressed the VLA-2 antigen which is a marker of chronic T cell activation. We further demonstrated that these $CD8^+/DR^+/CD25^-$ cells were specific for HIV in a CTL assay. Other studies demonstrated that $CD8^+$ cytotoxic T cells from AIDS patients gradually lost their HIV specificity over time in vivo and also in vitro upon restimulation with HIV antigens. Taken together, these observations may explain the gradual disappearance of HIV specific cytotoxic T cells over time in HIV infected individuals. (Koenig, Pantaleo, DeMaria, Wood, Fauci, LIR/NIAID; Moss, Earl, LVD/NIAID).

Clinical Investigation in AIDS

An ongoing study of 983 health care workers continues to demonstrate that the risk of nosocomial transmission of HIV is low, somewhere on the order of 0.4%.

Longitudinal followup of 20 individuals with indeterminate patterns on Western blot has revealed 2 individuals with true HIV-infection. The initial pattern on Western blot was not helpful in sorting out false positive from true positive and PCR positivity did not antedate antibody positivity by more than 3 months.

Immunologic evaluation of 50 patients with HIV-1 infection and pneumonitis revealed that the development of Pneumocystis carinii pneumonia was highly correlated with CD4 count. Patients with more than 250 CD4 cells/mm were at very low risk of developing this opportunistic infection.

A placebo-controlled trial of interferon-alpha (IFN-α) in HIV-1 infected individuals with more than 400 CD4 cells/mm^3 revealed a statistically significant lower rate of development of an AIDS defining opportunistic infection in the interferon treated group (0/17 vs 5/17).

Phase I studies were initiated of the following agents and combinations: 3'-azido-3'-dideoxyuridine (AZDU); AZDU + IL-2; IFN-α + IL-2. Phase II/III studies were initiated to examine the relative merits of zidovudine vs. IFN-α vs the combination in patients with HIV-1 infection and more than 500 CD4 cells/mm^3 and to examine the relative merits of zidovudine vs recombinant soluble CD4 vs the combination in patients with HIV-1 infection and 200-500 CD4 cells/mm^3.

A phase I trial of a candidate AIDS vaccine utilizing the HIV-1 envelope precursor glycoprotein, gp160 completed enrollment. Individuals immunized with up to 1280 ug of gp160 have developed antibodies and cell mediated immune responses to envelope proteins from a variety of HIV-1 isolates. (Lane, Fauci, R. Davey, Deyton, LIR/NIAID; Masur, Falloon, Kovacs, Polis, Henderson, CC/NIH)

International Studies on HIV Infection:

The AIDS continues to be a global pandemic with over 150,000 cases from 150 countries, and with an estimated 5-10 million infected individuals with HIV. International research efforts within LIR have been primarily focused on defining the unique epidemiologic, clinical, virologic, and immunologic features of AIDS in Africa and other tropical countries. At Project SIDA we were able to demonstrate a relatively high prevalence rate of 3.7% among 12,000 healthy employees at 2 large businesses at Kinshasa, Zaire and demonstrated that the major mode of transmission was heterosexual. Studies in this population, as well as among female prostitutes, demonstrated that heterosexual promiscuity and a history of genital ulcers were strongly associated with HIV transmission. Additional studies in Baltimore among patients attending sexually transmitted disease (STD) clinics and college students at the University of Maryland demonstrated that high risk behavior, such as multiple sexual partners and lack of use of condoms, continues in both populations, and that among STD patients, the single most important variable for infection with HIV was a recent episode of syphilis.

In our studies of perinatal transmission, we demonstrated that approximately 40% of children born to HIV infected mothers were infected perinatally, and that infection correlated with the clinical stage of HIV infection and state of immunocompetence in the mother at time of delivery, as measured by the number of CD4$^+$ cells and neutralizing antibody. We were able to demonstrate that symptomatic HIV seropositive subjects had higher titers of neutralizing antibody against HIV compared to asymptomatic individuals, and that neutralizing antibody and seropositive subjects in the Americas and Africa appear to have broadly cross-reactive neutralizing antibody against genetically divergent strains of viruses in the US, Haiti, and Africa. In studies of neurologic abnormalities in HIV infection, we demonstrated that p24 antigenemia and the level of antibody dependent cellular cytotoxicity within the cerebrospinal fluid correlated directly with advanced central nervous system (CNS) signs and symptoms of HIV infection, suggesting that the immune response to HIV infection may contribute to the pathogenesis of CNS HIV infection. Finally, we undertook a number of seroepidemiologic studies for human T lymphotropic virus-I (HTLV-1) infection. While rates of HTLV-1 infection remain high among patients residing in tropical areas such as pregnant women in Haiti (4.3%), prostitutes in Zaire (6.9%), HTLV-1 prevalence appears to be increasing in the US, particularly among STD patients (1.0%), inner-city minorities attending an emergency room department (1.2%), and recent prison inmates (1.0%). Preliminary neurologic studies in these patients demonstrate early neurologic manifestations, such as tropical spastic paraparesis, in these HTLV-1 infected patients. (Quinn, Brown, Fauci, LIR/NIAID; Ryder, CDC; Piot, Colebunders, Institute of Tropical Medicine, Antwerp, Belgium; Hook, Halsey, Johns Hopkins)

Isolation and Characterization of B Cell Specific Genes

The existence of various activation and functional stages in the progression of mature resting B cells to immunoglobulin (Ig) secreting cells implies that certain genes must be preferentially expressed during those various stages. Identification of these genes is likely to lead to important insights into the control mechanisms that are important in B cell function. We have initiated several projects to attempt to identify these important gene products. A cDNA library has been constructed from activated B cell mRNA and screened with B minus T cell mRNA probes to isolate gene products expressed in B cells and not T cells. Ten distinct cDNA clones are currently being studied. One of the clones, clone 66 has been fully sequenced and an open reading frame found which encodes for a 95 kd transmembrane protein. To date the mRNA has only been found to be expressed in B cells. The predicted protein is a member of the Ig superfamily and is partially homologous with the carcinoma embryonic antigen, myelin associated glycoprotein, and the PDGF receptor. The function of this protein is currently unknown although studies are in progress to address this issue. Several other clones have been partially sequenced and the sequencing of clone 11 will be completed soon. Additionally, 16 monoclonal antibodies have been isolated which were picked based on their preferential binding to B cells compared to T cells. These antibodies are being evaluated to determine if they recognize novel B cell surface antigens. (Kehrl, Rieckmann, Fauci, LIR/NIAID)

Regulation of Human B Cell Function by Soluble Factors

We have continued our studies on the sequencing and cloning of the high molecular weight B cell growth factor (HMW-BCGF) as well as the cellular receptor for this factor (BA5). Thus far, we have obtained reliable amino acid sequence of the 26 N-terminal amino acids of HMW-BCGF. In addition, reliable sequence was obtained for the N-terminus of both the intact 90 KD HMW-BCGF receptor and its 50 KD subunits. We are in the process of attempting to clone both.

We have continued our studies on the response to HMW-BCGF and the expression of receptors for this factor in B cells from patients with common variable hypogammaglobulinemia and in a variety of B cell leukemias in order to dissect out potential mechanisms of abnormal function in these cells. We have demonstrated that these cells are responsive to the factors in a manner discordant with the expression of receptors for the factors. This has allowed us to study and delineate a variety of post-receptor signal transduction defects in these conditions.

Collaborative studies have revealed that lymphocytes contain millimolar concentrations of vitamin C. The vitamin C is rapidly depleted when lymphocytes are placed into culture. Thus, in vitro lymphocyte cultures are performed with vitamin C deficient lymphocytes. The function of vitamin C in these cells remains to be elucidated but we have found evidence of one vitamin C dependent enzyme which is present in B cells as assessed by Northern blots.

Other studies have assessed the potential role of thyroid stimulating hormone (TSH) in B cell function. Activated B cells were found to express significant levels of TSH receptors as assessed by binding of biotinylated TSH and fluorescence-activated cell sorter (FACS) analysis. Functional studies have revealed only minimal effects of TSH on B cell proliferation and B cell differentiation to Ig secreting cells. In contrast to thyroid cells where TSH triggers a marked increase in cAMP levels no such increase is found in lymphocytes. Receptor crosslinking studies are in progress in order to characterize the receptor on B cells and thyroid cells. (Ambrus, Kehrl, Fauci, LIR/NIAID; Levine, Prabinkumar, NIDDK)

Effect of TGF-β on Lymphocyte Function

Our studies examining the effects of transforming growth factor-beta (TGF-β) on human immune function have continued. The TGF-β receptor on normal and transformed human B cells has been characterized by receptor crosslinking and receptor binding assays. The effects of TGF-β on B cell Ig transcription have been studied as have the effects of TGF-β on transcriptional regulatory factors important in B cell function. We have also initiated studies of the effects of TGF-β on kappa chain transcription in a pre-B cell line. During the course of these studies a phosphatase inhibitor, Okadaic acid, was found to induce kappa chain transcription in these cells and TGF-β was found to block this induction. We have also studied the effects of TGF-β on the HIV LTR CAT constructs and have found that Okadaic acid is a potent inducer of the HIV LTR in Jurkat T cells. This induction is also specifically blocked by TGF-β. In contrast, the activation of the LTR by tumor necrosis factor is not affected by TGF-β. Current studies are directed at examining the interaction between Okadaic acid and TGF-β in both the pre-B cell line and the Jurkat T cell line. (Kehrl, Rieckmann, Thevenin, Fauci, LIR/NIAID)

The Genetic Response of Primary T Lymphocytes to an Activation Signal: Control of Proliferation and Immune Competence

We have continued our studies on the elucidation of the primary genetic response of T lymphocytes to mitogenic activation. Using subtractive cloning technology we have identified previously in excess of 69 genes whose mRNAs are induced within a few hours of cellular stimulation. Included among the isolated genes were several known oncogenes, lymphokines and receptors, genes which play an important role in proliferation and the expression of a differentiated T cell phenotype. The many unknown genes obtained may perform similarly significant functions. We are pursuing both functional and regulatory aspects of our novel genes. Two such genes, AT 464 and AT 744, represent novel lymphokines which are regulated very similarly to IL-2 and which are sensitive to cyclosporin A. We determined their primary nucleotide sequence, we expressed them and we demonstrated their secretion from normal T cells by using specific peptide antibodies. These gene products are part of a superfamily of inflammatory mediators. Among our inducible genes we have discovered also a new member of the steroid receptor family and a zinc finger domain containing protein. The zinc fingers predict this protein to be a DNA binding protein which may be an important regulator of genes activated later in the cascade following mitogenic activation. Indeed, all HTLV-I transformed T cells tested to date constitutively express this gene. The HTLV-I virus is known to transactivate cellular genes, and this zinc finger gene is an excellent candidate for mediating transformation.

In addition we have continued our studies on the IL-2 promoter region which serves as a model system to dissect the regulatory components important for activating genes in T cells. We have identified previously several DNA binding proteins whose binding activity is induced during activation of the Jurkat T cells. One of these is an NF-kB like protein. We discovered that the NF-kB complex which forms with the HIV enhancer is activatable via distinct differentiable pathways. (Siebenlist, Zipfel, Schmidt, Burd, Wright, Wharton, Fauci, LIR/NIAID;

Kelly, LP/NCI; Greene, Duke University Medical School; Matsushima, FCRF/NCI; Levens, LP/NIC; McBride, DCBD/NCI)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

The LIR is currently studying prospectively one of the largest groups of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the last 21 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with corticosteroids. This therapy has dramatically altered the 5-year survival in patients with systemic vasculitis from 13% in untreated patients to 48% after treatment with corticosteroids to greater than 90% in our patients treated with both cyclophosphamide and corticosteroids. This therapeutic protocol was originally evaluated in our patients with Wegener's granulomatosis (WG), which now number over 150, but has been extrapolated and successfully used in treating other vasculitides such as polyarteritis nodosa, allergic angiitis and granulomatosis, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjogren's syndrome, and the polyangiitis overlap syndrome. Our therapeutic protocols for the vasculitic syndromes are now used worldwide. We have observed certain unexpected long-term side effects of cyclophosphamide and are currently developing new therapeutic protocols for the vasculitic syndromes. In this regard, we have begun to treat patients who cannot tolerate or fail to respond to cyclophosphamide therapy with cyclosporin A. Preliminary reports from other institutions have suggested that trimethoprim/sulfamethoxazole may be useful in the treatment of limited forms of WG. The anecdotal and uncontrolled nature of these observations has led us to initiate a prospective multicenter study to address this issue. We have also begun to study the efficacy and toxicity of monthly pulse cyclophosphamide in the vasculitic syndromes. Of 17 patients treated with "pulse" therapy, all have initially responded. However, six patients have had some form of disease recrudescence after one to eight months of treatment. In four cases disease exacerbation was modest. In two cases serious pulmonary relapses occurred. Follow-up periods range from 1-12 months.

Approximately 50% of patients with Takayasu's disease respond to corticosteroid therapy and about half of those who do not will respond completely to the addition of cyclophosphamide. The remainder generally continue to have smoldering disease. We have initiated a study to evaluate methotrexate, an immunosuppressive and anti-inflammatory agent, in the treatment of patients who have failed to achieve remission using corticosteroids, corticosteroids plus cyclophosphamide, or who have experienced cyclophosphamide toxicity that has precluded its continued use. Initial results in five patients suggest that methotrexate may be useful in treating Takayasu's arteritis when other approaches have failed. Follow-up on these patients ranges from 3 months to 2 years. Finally, we have been liberally employing surgical correction of stenotic lesions in patients with Takayasu's arteritis resulting in remarkably favorable functional improvement and survival rates. (Fauci, Leavitt, Hoffman, LIR/NIAID; Parrillo, Shelhamer, CC/NIH; Giordano, George Washington U. Medical Center)

Studies of the Immunopathogenic Features of Immune-Mediated Diseases

The immunopathogenic mechanisms of a number of immune-mediated diseases and/or diseases characterized by abnormalities of immune function were investigated. B cells from patients with common variable immunodeficiency (CVI) were studied and shown to respond normally to low molecular weight (LMW)-BCGF, but abnormally or not at all to HMW-BCGF despite the ability to express receptors for HMW-BCGF. This abnormality is compatible with a post receptor signal transduction defect. Our continuing studies of antineutrophil cytoplasmic antibodies (ANCA) in WG have demonstrated that ANCA has a high degree of specificity and sensitivity for WG. We have also demonstrated that bronchoalveolar lavage fluid (BAL) usually contains ANCA when serum is positive. The inversion of BAL albumin: gamma globulin ratios suggests that ANCA may be produced in the lung. We have been unable to demonstrate Ig deposition within the cytoplasm of freshly isolated peripheral blood neutrophils in patients with WG who are ANCA positive. This raises the question of whether exposure of immunocompetent cells to neutrophil antigen occurs at sites of inflammation, where neutrophil degranulation or lysis may occur. Our BAL studies have demonstrated that these latter events do occur in alveolar fluid. BAL cell populations were compared in normal control volunteers, patients with idiopathic pulmonary fibrosis (IPF) and WG. We have observed that a neutrophilic alveolitis occurs with exacerbations of WG. This contrasts with the usual increase in lymphocytes that has been noted in the alveolar fluid of patients having other granulomatous lung diseases, including sarcoidosis and tuberculosis. Fragmented and intact neutrophils were noted within alveolar macrophages in both WG, IPF and pneumonia; all of these disorders are associated with increased numbers of neutrophils in BAL fluid. Although normal controls rarely have greater than 3% neutrophils in alveolar fluid, this same observation was noted, although rarely, in this population. In conjunction with prior studies of alveolitis in rodents, our findings suggest that pulmonary macrophages phagocytose leukocytes as a means of limiting inflammatory injury to the lung. This represents the first demonstration of this phenomenon in man. (Fauci, Hoffman, Leavitt, Ambrus, LIR, NIAID; Sechler, Gallin, Strauss, LCI, NIAID; Baltaro, Fleisher, Shelhamer, Ognibene, Suffredini, CC, NIH; Wormsley, Peter, Specialty Laboratories).

Immunopathogenesis of Chlamydia Trachomatis

Chlamydia trachomatis continues to be the most common sexually transmitted bacterial pathogen in the United States with an estimate of approximately 5 million cases annually. The LIR has continued studies on the immunopathogenesis of C. trachomatis and the newly recognized pathogen, Chlamydia pneumoniae, one of the leading causes of pneumonia in the US. Clinical studies demonstrated high rates of infection of C. trachomatis (15-20%) in high association with human papillomavirus in women attending STD clinics in Baltimore. Infection during pregnancy was shown to be significantly associated with intrauterine growth retardation and pre-term delivery. Serotyping of chlamydia demonstrated that recurrences of chlamydia frequently occur with the same serovar particulary during coinfection with gonorrhea suggesting that microbiologically inapparent or persisting chlamydia infections may be rendered apparent or reactivated during recurring gonococcal infection.

During the past year we were able to synthesize oligonucleotide primers from published DNA sequences of the major outer membrane protein of C. trachomatis, thus enabling us to perform PCR for the detection of chlamydia genome. Utilizing these primers, we demonstrated that PCR was a highly sensitive and specific assay for the detection of chlamydia in human specimens. We plan to utilize PCR and our recently developed isotopic solution hybridization assay to study in more detail the immunopathogenesis of C. trachomatis and C. pneumoniae. (Quinn, LIR/NIAID)

Future Plans and Objectives

Over the past year, the LIR has made a number of significant contributions in the areas of the regulation of the human immune response in normal and disease states, the immunopathogenic mechanisms of infection with HIV, and clinical studies in AIDS.

We will further investigate the role of a variety of cytokines, including suppressive cytokines, in the regulation of HIV expression.

We will investigate whether cytokines other than tumor necrosis factor (TNF) can function in an autocrine/paracrine manner in the induction of HIV expression.

We will continue to investigate the molecular mechanisms of HIV induction by cytokines.

We will study the regulation of cytokine receptors and membrane associated cytokines (i.e., IL-1 and TNF) in HIV-infected cells.

We will pursue the study of the molecular mechanisms of induction of HIV expression by cell stress by exploring the possible involvement of heat shock proteins in this process.

We will pursue the mechanisms of infection of bone marrow progenitor cells with HIV.

We will continue studies on viral antigen-induced cytokines which induce HIV expression.

We will continue to use PCR to study prospectively the viral burden in purified CD4+ T cells from HIV-infected individuals, and determine its prognostic significance.

We will utilize PCR to study whether other putative cofactor viruses infect the same cells as does HIV.

We will pursue our studies involving the effects of colony stimulating factors on HIV-infected monocytes and attempt to delineate the mechanisms involved in GM-CSF inhibition of HIV infection of monocytes.

We will pursue the nature of HIV monocyte tropism by analyzing what molecular changes, if any, occurred when the molecularly cloned T cell adapted HIV isolate acquired an altered cell tropism after in vitro passage.

We will continue to study cellular and molecular changes in glial cells caused by HIV infection in the brain.

We will continue to study the function of GAP-43 in glial cells.

We will attempt to develop new experimental systems for the study of neuropathogenesis of HIV infection.

We will continue to map the specific epitopes within env, gag, pol, nef, tat, and rev of HIV that are recognized by HIV-specific cytotoxic T cells.

We will determine whether cytotoxic T cells specific for a particular HIV protein can prevent HIV replication in vitro or in an in vivo animal model.

We will determine whether subpopulations of CD8+, HIV-specific cytotoxic cells from seropositive individuals can be sufficiently expanded in vitro for use in in vivo reconstitution experiments.

We will be establishing models utilizing SCID and nu/beige/xid mice reconstituted with human peripheral blood from HIV-seropositive and seronegative donors to pursue questions related to pathogenesis of AIDS.

We will investigate the mechanisms responsible for the refractoriness of CD8+, DR+ T cells from HIV-infected individuals to in vitro proliferative signals.

We will attempt to determine the relationship between the expansion in vivo of the DR+, CD25- T cell population in HIV-infected individuals and the progression of disease.

We will investigate the mechanisms responsible for the progressive loss of HIV-specific cytotoxicity.

We will phenotypically characterize the effector cells involved in the immune response to HIV.

We will study the relative effects of HIV infection on naive versus memory T cells.

We will study the activation patterns of HIV-specific and hepatitis B virus T cell clones obtained by limiting dilution analysis after PHA activation relative to early and late activation antigens (CD25, DR, VLA, UCHL-1, LFAs, etc.) and to their functional status (proliferation, cytotoxicity, lymphokine production).

After determining the RNA structures critical for CAR function, we will elucidate the cellular proteins that interact with the CAR sequence to fully dissect the mechanisms of action of the rev protein. We will also investigate the effects of rev protein on cellular gene expression.

We will continue to evaluate the molecular mechanisms involved in the modulation of T cell activation in HIV-infected T cells.

We will continue to evaluate the role of gamma-delta T cells in the immunopathogenesis of HIV infection.

We will continue and expand our clinical trials program of anti-HIV drugs, drugs for the treatment of HIV infection-associated opportunistic infections, and immunomodulators.

We will continue our phase I trial of the gp160 vaccine candidate.

We will continue to analyze B cell specific gene products to obtain further insight into the molecular mechanisms of B cell function.

We will continue our studies of the mechanisms of gene expression associated with the activation of immune competent cells.

We will continue our study of the vasculitic syndromes and extend them by instituting alternative therapeutic protocols for the treatment of refractory disease as well as disease in patients who have developed prohibitive toxicity to our standard therapies.

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR have received a number of awards and honors.

Dr. Fauci became a member of the Committee for the Coordination of Research Programs on the Acquired Immunodeficiency Syndrome of the Ministry of Health of Italy and the Institute of Medicine Roundtable for the Development of Drugs and Vaccines Against AIDS. He continues to serve on a number of other committees of scientific and administrative importance.

Dr. Lane serves on two advisory boards to the Food and Drug Administration: the Office of Biologics - Immunomodulators, and the Office of Anti-Infectives - HIV Drugs. Dr. Quinn continues to be a member of the Scientific Advisory Committee to the Caribbean Epidemiology Center as well as a consultant to the Pan American Health Organization in the area of AIDS and sexually transmitted diseases. During the past year, he became a member of the U.S.-Japan Cooperative Medical Science Panel on AIDS and Other Human Retroviruses and the American Society for Clinical Research.

Dr. Fauci and other members of the LIR serve on editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. Dr. Fauci continues his role as an Associate Editor for the American Journal of Medicine. He is also on the Editorial Board of The Journal of Immunopharmacology, Clinics in Immunology and Allergy, EOS, Annals of Allergy, Physicians' Journal Update, Cellular Immunology, Immunopharmacology, The Journal of Molecular and Cellular Immunology, AIDS Research and Human Retroviruses, and AIDS Patient Care. He serves on the Advisory Board of Clinical Immunology and Immunopathology and the Journal of Clinical Immunology. He is on the Editorial Advisory Council of La Ricerca in Clinica e in Laboratorio, the International Scientific Committee of Immunologia Clinica e Sperimentale, and is Consulting Editor for North America for the journal Thymus.

Dr. Fauci continues as an editor of "Harrison's Principles of Internal Medicine," "Current Therapy in Allergy, Immunology and Rheumatology," and "Harrison's Principles of Internal Medicine - Companion Handbook." He is an associate editor of "Current Therapy in Internal Medicine." In addition, he continues to edit with Dr. John I. Gallin "Advances in Host Defense Mechanisms."

Dr. Quinn is a member of the editorial board for Sexually Transmitted Diseases, AIDS, AIDS Research and Human Retroviruses, Journal of the Acquired Immunodeficiency Syndrome, and the International Journal of STD and AIDS. Dr. Lane serves on the editorial boards of the Journal of Clinical Immunology, Journal of the Acquired Immunodeficiency Syndrome, AIDS Research and Human Retroviruses, and Clinical Immunology and Immunopathology.

Dr. Fauci as well as several members of the LIR were asked to deliver major named or invited lectureships during the year. He was the Mr. and Mrs. I. G. Bromberg Visiting Professor at the Department of Medicine of the University of Texas at Dallas Health Science Center. Dr. Fauci was also an invited speaker at the National Academy of Science President's Circle on Issues Confronting the Global Future, an invited plenary speaker at the Annual Meeting of the Association of American Medical Colleges, and an invited symposium speaker at the First Forum on AIDS of the Harvard AIDS Institute. Dr. Fauci was also an invited plenary lecturer at the Ninth European Immunology Conference in Rome, the International Symposium on AIDS in the Republic of San Marino, the U. S. Health Summit on HIV Infection, and the Georgetown University Bicentennial Symposium. He delivered a state of the art Lecture at the Annual Meeting of the American College of Rheumatology. Dr. Fauci also delivered keynote addresses at the Advances in Pediatric Education and Scientific Forum of the Children's Hospital National Medical Center, the Annual Meeting of the American Academy of Physician Assistants, the American Association for the Advancement of Science 1989 Mass Media Science and Engineering Fellows Program, the Annual Meeting of the American Society for Medical Technology, and the HIV Treatment Awareness Week in San Francisco.

Of particular note, Dr. Fauci was the Distinguished Scientist Lecturer at the Bard College Lecture Series. He also delivered the C. Richard Bowman Memorial Lectureship of the University of Virginia School of Medicine, the 54th Annual William Beaumont Society Lectureship of the George Washington University School of Medicine, the First Annual William D. Dolan, M.D. Lecture of the Arlington Hospital Foundation, and the Maurice C. Pincoffs Lectureship in Medicine at the University of Maryland School of Medicine.

Finally, Dr. Fauci delivered the commencement address at The Mount Sinai School of Medicine, The Johns Hopkins School of Medicine, and The University of Michigan School of Medicine.

During the past year, Dr. Koenig was an invited lecturer at several national and international meetings including the Third "Cent Gardes" Meeting on Retroviruses of Human AIDS and Related Animal Diseases in Paris, MRC Directed Meeting on Cell Mediated Immunity in Cambridge, England, the Center for Nonlinear Studies and Sante Fe Institute Workshop on Modeling the Interaction of HIV with the Immune System, and at the annual meetings of the American College Health Association and the American Society of Pathology.

Dr. Lane was a visiting professor at Western Pennsylvania Hospital, and invited symposium speaker and session chairperson at the V International Conference on AIDS.
Dr. Vitkovic delivered an invited lecture at the International Symposium on Brain Damage and Plasticity in Kogor, Yugoslavia, and at the V International Conference on Neurologicaland Neuropsychological Complications of HIV Infection in Quebec. Dr. Siebenlist was a workshop chairman at the 7th International Congress of Immunology in Berlin. Dr. Quinn was an invited plenary speaker at the American Society for Academic Emergency Medicine and was a visiting professor at the University of North Carolina, Chapel Hill, and the University of Florida, Gainesville. Dr. Hoffman was an invited lecturer during the Holland Digestive Disease Week, which celebrated the 75th anniversary of the Netherlands Society of Gastroenterology

Finally, over the past year, Dr. Fauci received the American Association for the Advancement of Science/Westinghouse Award for Public Understanding of Science and Technology, the National Hemophilia Foundation Special Award for Research in AIDS, the Lee P. Brown National Public Service Award presented by the National Academy of Public Administration and the National Society for Public Administration, the Duke University Award for Rheumatologic and Immunologic Research, the William Beaumont Award of the American Medical Association, the Academic Excellence Award from Children's Hospital National Medical Center, the Surgeon General's Exemplary Service Medal and the Surgeon General's Medallion, and the Leadership Award of the Columbus Citizens Foundation, Inc., in New York City.

Dr. Quinn was honored by receiving the U.S.P.H.S. Outstanding Service Award.

Administrative, Organizational, and Other Changes

The major theme of the LIR continues to be the delineation of the mechanisms of regulation of the human immune response in normal and disease states. The vast majority of activities of the LIR now focus on AIDS, particularly the study of the immunopathogenic mechanisms of HIV infection. Following the Congressional and administrative mandates to increase AIDS activities at the NIH, the LIR has expanded modestly.

Dr. H. Clifford Lane has assumed leadership of the Clinical and Molecular Retrovirology Section of the LIR and has expanded the vaccine and therapeutic clinical trials program of the laboratory. Dr. John Zurlo has joined the clinical studies component of the section, and Dr. David Cohen has joined the molecular retrovirology component. Dr. Lawrence Deyton has left the group to join the Division of AIDS of NIAID where he will direct the Community Program for Clinical Research on AIDS.

Studies on the immunopathogenic mechanisms of HIV infection have expanded over the past year. Dr. Guido Poli has advanced from a Visiting Fellow to a Visiting Scientist and will remain in the LIR for at least two to three more years. Drs. Guiseppe Pantaleo and Andrea DeMaria have joined the group working directly with Dr. Fauci. Dr. Luca Butini has recently arrived and will be working initially with Dr. Pantaleo. Dr. Tea Kalebic and Anna da Cunha have joined Dr. Ljubisa Vitkovic's unit. Drs. Peter Rieckmann and Claire Thevenin have joined Dr. John Kehrl's unit as guest researchers. Drs. Parris Burd and John Wright have joined Dr. Uli Siebenlist's molecular biology unit. Dr. Lauren Wood has been with the LIR for one year and will continue on for at least one more year.

Dr. Julian Ambrus has left the LIR after seven years to join the faculty at Washington University in St. Louis. Dr. Ferdinand Massari has finished his fellowship and will work for Merck Co. Drs. Peter Zipfel and Albrecht Schmidt have completed their post-doctoral fellowships with Dr. Siebenlist and have returned to West Germany to medical faculty positions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00210-09 LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation of Human Lymphocyte Function in Normal and Disease States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	J.H. Kehrl	Senior Investigator	LIR, NIAID
	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	U. Siebenlist	Senior Staff Fellow	LIR, NIAID
	P. Bressler	Medical Staff Fellow	LIR, NIAID
	P. Rieckmann	Guest Researcher	LIR, NIAID
	C. Trevenin	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

BRB, NIAID (J. Coligan); LI, NIAID (T. Chused); Washington Univ. (E.J. Brown, M. Peters); Duke Univ. (W.C. Greene, M. Dukovich, R. Buckley); NCI (S. Rudikoff, A. Roberts, M. Sporn).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL.	OTHER
7	4	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the mechanisms of B cell activation, proliferation, and differentiation at the molecular and cellular levels. Using subtractive hybridization techniques we have isolated several novel cDNA clones which are differentially expressed between B and T lymphocytes. Two of the cDNA clones have been fully sequenced, the predicted amino acid sequence analyzed and used for the synthesis of synthetic peptides for antibody production. Northern blot analysis of several of the B cell specific clones have revealed variable expression during B cell activation and variable expression in different B cell lines. During the characterization of the subtracted clones a full length cDNA clone for the B1 molecule was isolated. We used this clone to isolate 3 genomic clones which encompasses the entire B1 gene. One of the genomic clones was used to isolate the promoter for this gene and to make promoter-CAT constructs for transfection experiments. We have continued our studies of the effect of transforming growth factor-β (TGF-β) on human B cell function and have made use of the murine 70 Z pre-B cell line. The effects of TGF-β on various transcriptional regulatory factors important in B cell Ig production have been examined. We have shown that Okadaic acid is a potent inducer of kappa light chain expression in 70Z cells and that TGF-β markedly inhibits its induction. Studies are in progress to examine the mechanisms by which Okadaic acid induces kappa gene expression. Studies of the TGF-β promoter have revealed that both TAT and TAX can upregulate the promoter in Jurkat T cells. Studies are in progress to examine the transcriptional regulatory factors present in human T and B cells. We have continued our studies on the sequencing of the high molecular weight B cell growth factor (HMW-BCGF) as well as the cellular receptor for this factor (BA5) and are attempting to clone the genes for both.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00212-09 LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of the Immunopathogenic Features of Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	G.S. Hoffman	Expert	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH, (J.E. Parrillo); Mayo Clinic, Rochester, MN, (G. Gleich); Univ. Minnesota, (F. Uckun); Duke Univ., (R. Buckley); Georgetown Univ. (P. Katz, T. Cupps); SUNY, Buffalo, (D. Farolino, G. Logue); Speciality Laboratories, (J. Peter, S. Wormsley); LPD, NIAID (D. Kaslow).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunopathogenic mechanisms of a number of immune mediated diseases and/or diseases characterized by abnormalities of immune function were investigated. B cells from patients with common variable immunodeficiency (CVI) were studied and shown to respond normally to low molecular weight B cell growth factor (LMW-BCGF), but abnormally or not at all to high molecular weight (HMW)-BCGF despite the ability to express receptors for HMW-BCGF. This abnormality is compatible with a post receptor signal transduction defect. Indeed, our data suggest that overproduction of an arachidonic acid metabolite such as PGE or one of the leukotrienes may be playing an inhibitory role in the normal signal transduction occurring with HMW-BCGF. Use of the lipoxygenase inhibitors ketoprofen or hydrocortisone have resulted in markedly enhanced production of antigen specific antibody in vitro. We have extended our studies on the hypereosinophilic syndrome (HES) and have recently had the opportunity to investigate a large family cohort of patients with hereditary hypereosinophilia. They will serve as a valuable model for study of this interesting syndrome. We have demonstrated the presence of antineutrophil cytoplasmic antibodies (ANCA) in patients with Wegener's granulomatosis, lymphomatoid granulomatosis, polyarteritis nodosa, and systemic lupus erythematosus (SLE). In some cases, the ANCA IgG correlates with disease activity. The data indicate that ANCA is a sensitive and specific marker for Wegener's granulomatosis. Finally, we have continued our studies of idiopathic dilated cardiomyopathy and have demonstrated that this disease is responsive to immunosuppressive therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00213-09 LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	G.S. Hoffman	Expert	LIR, NIAID

COOPERATING UNITS *(if any)*

Critical Care Medicine Department, Clinical Center, NIH (J.E. Parrillo, J. Shelhamer, F. Ognibene) Clinical Pathology, Clinical Center, NIH (T. Fleisher, R. Baltaro); Laboratory of Clinical Investigation (J. Sechler, J. Gallin); George Washington University Medical Center (J. Giordano).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The LIR is currently studying prospectively one of the largest groups of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the last 24 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. On the basis of our observations, lymphomatoid granulomatosis is classified as a subset of malignant lymphoma and not as a primary vasculitis. We have described a new vasculitic syndrome termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with corticosteroids. This therapy has dramatically altered the 5-yr survival in patients with systemic vasculitis from 13% in untreated patients to 48% after treatment with corticosteroids to greater than 90% in our patients treated with both cyclophosphamide and corticosteroids. This therapeutic protocol was originally evaluated in our patients with Wegener's granulomatosis which now number over 175, but has been extrapolated and successfully used in treating other vasculitides such as polyarteritis nodosa, allergic angiitis and granulomatosis, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjogren's syndrome, and the polyangiitis overlap syndrome. Our therapeutic protocols for the vasculitic syndromes are now used worldwide. We have begun to study the efficacy and toxicity of monthly pulse cyclophosphamide in the vasculitic syndromes. We are also treating eligible patients with Wegener's granulomatosis with trimethoprim-sulfamethoxazole in order to evaluate its efficacy in treating this disorder. In addition, patients with Takayasu's arteritis who are not controlled with corticosteroid therapy are being studied on a treatment protocol evaluating methotrexate therapy. Hormone studies are being performed in patients with Takayasu's arteritis in order to assess the role of estrogens in the pathogenesis of the disorder. Studies of bronchoalveolar lavage fluid from patients with Wegener's granulomtous have revealed a predominance of neutrophils which is distinct from the predominance of lymphocytes seen in other diseases causing granulomatosis inflammation in the lung.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00390-06 LIR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Investigation of the Acquired Immunodeficiency syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.C. Lane	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	R.T. Davey	Medical Officer	LIR, NIAID
	K.M. Zunich	Medical Officer	LIR, NIAID
	M. Easter	Nurse Practitioner	LIR, NIAID
	D. Lee	Project Coordinator	LIR, NIAID
	V. Davey	Project Coordinator	LIR, NIAID

COOPERATING UNITS (if any)

CC, NIH (H. Masur, J. Kovacs, J. Falloon, M. Polis, D. Henderson)

LAB/BRANCH
Laboratory of Immunoregulation

SECTION
Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An intensive effort was directed toward studying the epidemiologic, virologic, immunologic and therapeutic aspects of the acquired immunodeficiency syndrome. An ongoing study of 983 health care providers has continued to demonstrate the low risk of human immunodeficiency virus-1 (HIV-1) transmission from patient to health care worker with a seroconversion rate of 0.4% per percutaneous exposure. An 18 month follow-up of twenty individuals with indeterminate patterns on HIV Western blots failed to reveal a pattern indicative of early exposure and demonstrated a less than 3 month interval between polymerase chain reaction positivity and full seroconversion. A subset of patients with HIV-1 infection were found to have increased numbers of CD8+ gamma-delta cells in the peripheral blood. A cell line was isolated from a patient with HIV-1 infection that coexpressed CD4 and CD8, harbored inducible HIV-1 and expressed a new interleukin-2 (IL-2) receptor. Immunization of healthy human volunteers to the gp160 envelope precursor protein of HIV-1 was capable of inducing antibody and T lymphocyte blast transformation responses with group specificity to both recombinant and natural product HIV-1 proteins. Clinical trials were completed that demonstrated the potential value of zidovudine (ZDV) and interferon-α (IFN-α) either alone or in combination in the treatment of early stage HIV-1 infection. Clinical trials were initiated to compare the effects of ZDV and IFN-α alone or in combination in patients with early HIV infection and to compare the effects of ZDV and rsCD4 either alone or in combination in patients with mid-stage HIV infection. Phase I studies were initiated to evaluate AZDU, AZT + IFN-α + GM-CSF, ZDV + IL-2 and IFN-α +IL-2 in patients with HIV-1 infection. Unsuccessful attempts at cellular immune reconstitution examined the effects of ZDV in combination with bone marrow transplantation and syngeneic lymphocyte infusions.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00431-05 LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biologic Approach to the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	U.K. Siebenlist	Senior Staff Fellow	LIR, NIAID
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	J. Wright	Guest Researcher	LIR, NIAID
	P.F. Zipfel	Guest Researcher	LIR, NIAID
	A.G. Schmidt	Guest Researcher	LIR, NIAID
	P. Burd	National Research Services Fellow	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

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LP/NCI (Kelly, Levens); FCRF/NCI (Matsushima); Duke Univ. Med. School (Greene); DCBD/NCI (McBride)

LAB/BRANCH

Laboratory of Immunoregulation

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INSTITUTE AND LOCATION

NIAID, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied the primary genetic response of human peripheral blood T cells to mitogenic activation. By employing subtractive cloning technology, have previously isolated in excess of 69 genes whose expression is induced within hours of cellular stimulation. Included among these genes are several well-known lymphokines, receptors and oncogenes in addition to many novel genes whose functions are suspected to be similarly important for proliferation and the expression of a differentiated T cell phenotype. We have pursued both functional and regulatory aspects of the novel genes. Two genes selected for detailed studies encode novel lymphokines which are likely to play a role during inflammatory reactions. We have expressed these genes and have obtained peptide antisera as well as recombinant material. We have cloned and sequenced their promoters for regulatory studies, including the inhibitory effect exerted by cyclosporin A. Two further genes selected for detailed studies encode a new member of the steroid receptor family and a very highly inducible zinc finger protein. The presence of zinc fingers suggests a potential role as a DNA regulatory factor which is supported by the discovery that HTLV I transformation of T cells leads to constitutive expression of this gene. Indeed all HTLV I or HTLV II transformed cells tested to date express this gene. We have dissected further the activation responsive DNA elements and DNA binding proteins in the IL-2 model system. One such protein is an NF-KB like protein and we have discovered that the NF-KB complex interacting with the HIV enhancer can be activated via distinct signal pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-07 LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of Chlamydia trachomatis infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins University, (E. Hook, S. Holland)

LAB/BRANCH

Laboratory of Immunoregulation

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the US with an estimated 5 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infection in experimental animal models. In women attending sexually transmitted disease clinics, chlamydia cervical infections continue to be high (20-30%). Coinfection with gonorrhea and human papillomavirus (HPV) frequently occur and are strongly associated with the appearance of cervical dysplasia and cervical carcinoma. In a study of 116 consecutive women attending STD clinics, a cumulative prevalence of HPV infection was 49%, and 63% had additional coinfections, suggesting that coinfections with HPV may play an important role in inducing cervical dysplasia. In a study of 801 pregnant women, chlamydia was present in 15.5% of the patients and was significantly associated with intrauterine growth retardation and with preterm delivery. Serotyping of chlamydial clinical isolates has been developed in our laboratory and the most common serovars in women were D (26%), E (22%), and F (25%). Patients with recurrent gonorrhea were significantly more likely to experience recurrence of chlamydia due to the same serovar, suggesting that microbiologically inapparent or persistent chlamydial infections may be reactivated during recurrent gonococcal infection. Diagnostic assays including in situ hybridization and polymerase chain reaction (PCR) have been developed, and a high sensitivity and specificity for detecting chlamydia in tissue samples has been documented. Utilizing three sets of oligonucleotide primer pairs from the major outer membrane protein of C. trachomatis, it was possible to detect all 15 serovars of C. trachomatis and to differentiate between C. trachomatis, C. pneumoniae, and C. psittaci. Experimental animal inoculations with C. pneumoniae demonstrated establishment of mucosal infections of the eye, nasopharynx and intestine of cynomolgus monkeys, followed by a serologic immune response. Further studies are planned to examine the clinical spectrum of serovar-specific chlamydial infections and the immunopathogenesis of C. pneumoniae in non-human primate models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00361-07-LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Studies on the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
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	C. Brown	Expert	LIR, NIAID
	R. Kline	Microbiologist	LIR, NIAID

COOPERATING UNITS *(if any)*

CDC (R. Ryder); Institute of Tropical Medicine, Antwerp, Belgium (P. Piot, R. Colebunders); Johns Hopkins University (E. Hook, N. Halsey)

LAB/BRANCH

Laboratory of Immunoregulatioan

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The acquired immunodeficiency syndrome (AIDS) has become a global pandemic with over 150,000 cases reported from 150 countries. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic, and immunologic features of HIV infection in Africa and other tropical countries. In a survey of 12,032 employees and their spouses at 2 large businesses in Kinshasa, Zaire, 3.7% were antibody positive for HIV, and the predominant mode of transmission was via heterosexual transmission. In this study AIDS was found to be the leading cause of death among newly employed individuals. We also demonstrated that 37% of 841 female prostitutes in Kinshasa, Zaire were HIV seropositive and that genital ulcerations were strongly correlated with transmission. The role of sexually transmitted diseases (STD's) as cofactors in HIV transmission was further supported by our recent study that demonstrated that 4.9% of 4,863 STD patients in Baltimore were HIV positive and that the single most important variable for infection was a recent episode of syphilis. In a study of perinatal transmission, we demonstrated that approximately 40% of children born to 600 HIV infected women in Zaire and Haiti were infected perinatally. Clinical stage of HIV infection, declining neutralizing antibody and depressed CD4+ cells in the mother correlated with perinatal HIV transmission. During several immunologic studies we demonstrated that increasing antibody dependent cellular cytotoxicity (ADCC) and p24 antigenemia in the cerebrospinal fluid correlated with advanced neurologic disease in HIV infection. HIV antigenemia and circulating levels of tumor necrosis factor appeared to be depressed in African AIDS patients suggesting either increased antigen-antibody complexing or a chronically immune activated state. Serologic screening for HTLV-1 demonstrated a 6.9% infection rate among Zairian prostitutes, 4.3% of pregnant Haitian women, 1% of STD patients in the U.S., 1.2% of patients in an adult emergency department in Baltimore, and 1% of prison inmates in Philadelphia, demonstrating increasing prevalence of this additional human retrovirus. Further studies will continue to examine the immunopathogenesis and natural history of HIV-1, HIV-2, and HTLV-1 in selected populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00537-01 LIR

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunopathogenic Mechanisms of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
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	S. Koenig	Senior Staff Fellow	LIR, NIAID
	S. Schnittman	Medical Staff Fellow	LIR, NIAID
	G. Poli	Visiting Associate	LIR, NIAID
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	S. Stanley	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Immunoregulation

SECTION

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
20	13	7

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The LIR has been intensively involved in the delineation of the cellular and molecular mechanisms of the immunopathogenesis of human immunodeficiency virus (HIV) infection. Using our previously developed chronically HIV-infected, cloned T cell (ACH2) and promonocytic (U1) lines we have demonstrated that tumor necrosis factor-α (TNF-α) alone is capable of inducing the expression of HIV in ACH2 while in addition to TNF, interleukin-6 (IL-6) and granulocyte macrophage-colony stimulating factor (GM-CSF) alone or in synergy with TNF can induce HIV expression in U1 cells. This is of relevance since these cytokines are important physiologic cytokines involved in immunoregulation and TNF levels are elevated in the plasma of HIV infected individuals. We demonstrated that TNF induces HIV expression in ACH2 cells through the induction of transcription activating factors that bind to the NF-kB sequences in the HIV long terminal repeat. Furthermore, we clearly demonstrated that TNF can function in an autocrine manner in the induction of HIV expression. Heat shock can induce expression of HIV in our cell lines and transient temperature elevations to 40°C can synergize with TNF and IL-6 in the induction of HIV. We demonstrated that the primary reservoir of HIV in the peripheral blood is the CD4$^+$ T cell which maintains its expression of CD4 and the frequency of CD4$^+$ T cells containing HIV provirus is greater than 1:100 in AIDS patients. We cloned HIV-specific, major histocompatibility complex restricted T cell clones from the peripheral blood of HIV seropositive individuals and have begun to map the epitopes within env, gag, and nef proteins of HIV which are recognized by cytotoxic T cells. We have demonstrated a defective clonogenic potential of CD8$^+$, DR$^+$, HIV specific cytotoxic T cells in AIDS patients. This may explain the progressive disappearance of HIV-specific cytotoxic T cells in HIV infection. We are elucidating the structure critical to the function of CAR which is the target sequence for the rev regulatory gene of HIV.

Others:	G. Pantaleo	Visiting Fellow	LIR, NIAID
	A. De Maria	Guest Researcher	LIR, NIAID
	E. Duh	Guest Researcher	LIR, NIAID
	L. Wood	NRSA	LIR, NIAID
	C. Fox	Medical Officer	LIR, NIAID
	A. Dayton	Senior Staff Fellow	LIR, NIAID
	E. Dayton	Staff Fellow	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID

Cooperating Units: PRI, Fredrick (M. Baseler, N. Young); Harvard Children's Hospital (H. Winter); The Brigham and Women's Hospital (A. Kiesling); NYU Medical Center, Hospital for Joint Diseases (S. Itescu), UCLA Med. Center (J. Bernstein), Northwestern Univ. (S. Wolinsky), Johns Hopkins Univ. (E. Hook, J. Tunkel).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00562-01 LIR

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Neuroimmunology and Neuropathogenesis of HIV Infection

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	L. Vitkovic	Expert	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
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	A. daCunha	Visiting Fellow	LIR, NIAID
	H. Ehrenreich	Guest Researcher	LIR, NIAID

COOPERATING UNITS (*if any*)

BRB, NIAID (J. Coligan); LN, NINDS (M. Brightman); Med. Col. Penn., Phil. PA. (V. Aloyo); INSERRM Unite 44, Strasbourg, France (M. Mersel, N. Neskovic).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS 4	PROFESSIONAL 3	OTHER 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We have initiated studies on the role of macroglial cells in the neuropathogenesis of human immunodeficiency virus (HIV) infection. Using human promonocytic cell clones chronically infected with HIV, we demonstrated that astrocytes stimulated with lipopolysaccharide secrete factors which induce HIV. One of the factors was identified by multiple criteria, as tumor necrosis factor alpha (TNFα). In an effort to identify other factors we found that astrocytes constitutively synthesize, at a low level, mRNA encoding transforming growth factor beta (TGF_{β}). Results indicate that TNFα may be toxic for type 2 astrocytes in addition to its reported toxicity for oligodendrocytes. We have determined approximately 8% of the amino acid sequence of UDP galactose : ceramide galactosyltransferase (CgalT) an enzyme involved in myelination and synthesized by oligodendrocytes. We have perviously demonstrated that a major membrane-associated protein kinase C substrate in brain, growth-associated protein 43 (GAP-43), is present in astrocytes. We have demonstrated that it is also present in oligodendrocytes and in both types of macroglial progenitors. Results indicate that GAP-43 expression is differentially regulated: type 1 astrocytes down-regulate GAP-43 whereas type 2 astrocytes and oligodendrocytes express it constitutively.

LID

LABORATORY OF INFECTIOUS DISEASES
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SUMMARY STATEMENT
Annual Report
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1988 to September 30, 1989

INTRODUCTION

Scientists in LID continue to pursue the long term goals of: (a) delineating the etiology, pathogenesis and epidemiology of medically important viral diseases, (b) defining the fine structure, genome organization and antigenic composition of the most consequential viral pathogens and (c) developing means for treating or preventing these diseases. Significant success was achieved in each of these areas of research during the past year.

Hepatitis and AIDS Viruses

Hepatitis A Virus (HAV). RNAs transcribed from cDNA clones of wild type or tissue culture adapted HAV or mutants derived from these clones were analyzed in an in vitro translation system to gain a better understanding of genome organization and gene boundaries. Using a newly developed mutagenesis system the first HAV gene (capsid protein VP4) was deleted and replaced by poliovirus VP4. Translation of RNAs from the chimeric construct yielded chimeric polyproteins recognized by antipeptide antibodies to poliovirus VP4 or HAV VP2 capsid protein.

Genetic changes in the genome of HAV responsible for adaptation to growth in cell culture were mapped by analyzing the properties of viruses derived from infectious chimeric cDNAs which contained varying portions of the genomic sequence of wild type virus (non-cultivable, virulent) and its tissue culture passaged mutant (cultivable, attenuated). The mutations responsible for growth of virus in cell culture appeared to be confined to two genes (2B and 2C) in the P2 non-structural protein region of the genome. Site-directed mutagenesis was used to change individual nucleotides in the P2 region of the tissue culture-adapted, attenuated cDNA clone to wild type sequence. Analysis of the resulting constructs in tissue culture indicated that not more than 4 mutations, and possibly as few as 2 mutations in the 2B and 2C genes of wild type virus were sufficient to effect efficient growth of HAV in cell culture.

Considerable progress was achieved in our effort to develop a satisfactorily attenuated and effective live HAV vaccine. The attenuated strain of HAV initially adapted to primary African green monkey kidney tissue culture in LID was adapted to growth in a cell line suitable for use in production of human vaccines. A suspension of this virus prepared and safety tested by Smith-Kline-RIT, was shown to be completely attenuated and immunogenic in chimpanzees. Significantly, chimpanzees previously infected with the candidate HAV vaccine strain were completely protected against hepatitis following challenge with 10^3 infectious doses of HAV. Clinical trial of this candidate vaccine should begin soon.

Hepadnaviruses. These viruses possess a small circular double stranded DNA genome (approximately 3000 nucleotides in length) which encodes an unusually large amount of protein. This is achieved through the use of overlapping reading frames. Triple utilization of a genome sequence, although rare in genetics, is employed in many regions throughout the hepatitis B virus (HBV) genome.As a consequence cis-acting elements are located within regions which also encode proteins. Highly conserved genomic domains were identified by sequence alignment of 14 hepadnavirus genomes and these invariant regions were tentatively identified as the location of cis-acting regulatory regions including promoters, an enhancer, a poly-A addition signal and a glucocorticoid responsive element.

The woodchuck hepatitis virus (WHV) is closely related to HBV and represents the most meaningful experimental surrogate for HBV. Recombinant WHV DNA was shown to be infectious for woodchucks following transfection of liver in vivo. This important observation sets the stage for genetic studies with WHV which were not possible until now because the virus has not been grown in cell culture. For example, in vivo transfection analysis of WHV DNA with deletions of varying size in the X protein gene indicated that this region of the viral genome, whose function has not been defined as yet, is essential for viral replication and/or gene expression.

Finally, the polymerase chain reaction (PCR) assay was shown to be 10^4 fold more sensitive than previous techniques for detection of HBV DNA in the serum of patients with HBV chronic hepatitis. Using this technique it is now possible to detect as few as 3 HBV DNA genomes per 10 l of serum. PCR detected HBV DNA in the serum of patients with chronic hepatitis who had developed antibodies to HBsAg, indicating that, contrary to prevailing opinion, this type of seroresponse does not signal termination of viral replication.

New hepatitis viruses. In 1989 scientists in Canada proposed that giant cell hepatitis, a common cause for liver failure in the neonatal period, is caused by a paramyxovirus. As a consequence, collaborative studies of this disease have been initiated, including attempts to transmit hepatitis to primates.

Experimental studies of simian and feline AIDS. A new research program was recently initiated in LID to study two groups of lentiviruses which cause AIDS-like disease in their natural host. Two experimental systems were established: (1) a primate model for the study of simian immunodeficiency virus (SIV) and (2) a feline model for the study of feline immunodeficiency virus (FIV).

Strains of SIV and FIV have been cloned and completely sequenced and it is clear that both are lentiviruses. Rhesus monkeys have been successfully infected with SIV and a proportion of infected animals have developed an AIDS-like disease and died. Cats have also been infected with FIV but disease has not developed to date. Molecular clones of SIV and FIV have been recovered which are infectious for both cell culture and the appropriate animal host.

Four distinct subtypes of SIV have been delineated: (1) SIVmac from rhesus monkeys; (2) SIVsmm from sooty mangabeys; (3) SIVagm from African green monkeys and (4) SIVmnd from mandrills. Extensive genetic heterogeneity was demonstrated among strains of SIVagm. In fact divergence between 2 strains of SIVagm was as great as that observed between SIVmac and HIV-2. Surprisingly, SIVsmm was found to be more closely related to HIV2 than was SIVagm. SIVmac was also closely related to SIVsmm. Because infection of rhesus monkeys with SIVmac has not been detected in the wild, whereas, sooty mangabeys are commonly infected with SIVsmm in nature, it appears that (1) SIVsmm was the source of SIVmac via cross-species transmission in a primate facility and (2) SIVsmm or its immediate ancestor may have entered the human population in West Africa (where sooty mangabeys are indigenous) and evolved into HIV-2.

Extensive genetic variation and resultant heterogeneity of SIVsmm was observed during infection of rhesus monkeys. Multiple pro-viral forms of SIV were observed to emerge during infection and certain of these variants could not be recovered in tissue culture despite a high copy number in vivo. The significance of these heterogeneous genomes is now under active investigation in monkeys infected with: (1) a heterogeneous population of uncloned virus particles (simulating what happens under natural conditions of infection) or (2) a relatively homogeneous virus suspension derived from an infectious molecular clone.

Molecular analysis of FIV indicates that it is a bona fide lentivirus. The availability of an infectious molecular clone of FIV should facilitate a detailed inquiry into the molecular pathogenesis of feline immunodeficiency disease.

Dengue Viruses

Dengue viruses continue to pose a major threat to human health in most tropical and semitropical areas. Unfortunately safe, effective vaccines are not available for these viruses which are the most frequent cause of mosquito-borne viral disease.

Processing of viral polyprotein. The 10 Kb viral genome (positive strand RNA) codes for a large polyprotein which is processed into at least 10 distinct proteins. More than 75% of the viral genome codes for the 7 known non-structural proteins. The first of these (coded by the 4th viral gene) is NS1, a glycoprotein which is displayed in low copy number on the surface of infected cells where it serves as a target for NS1-specific cytolytic, protective antibodies. Processing of NS1 from the polyprotein was analyzed using a series of vaccinia virus-dengue NS1-NS2a recombinants. In these studies the downstream protein NS2a was shown to be required in cis for cleavage of the NS1/NS2a junction. All but the 20 C-terminal amino acids of NS2a were required for proper cleavage. Similar analysis of the processing of NS3, tentatively identified as a viral RNA polymerase, indicated that the upstream NS2a-NS2b region is required for proper cleavage at the NS2b/NS3 and NS3/NS4a junctions.

Processing of viral glycoproteins. The second, third and fourth genes in the viral genome each encode a glycoprotein. Thus, in the polyprotein the non-glycosylated capsid (C) protein is followed by the pre-M, envelope (E) and NS1 glycoproteins in that order. Cleavage at the C-preM and preM-E junctions is mediated by signal peptidase and is linked to translocation into the endoplasmic reticulum. Translocation appears to be mediated by a hydrophobic domain located at the C-preM junction.

Protective antigens. The E and NS1 glycoproteins were identified as independent protective antigens during studies in which these glycoproteins, expressed singly or together by a baculovirus (b) or vaccinia (v) virus recombinant, were evaluated for their capacity to induce resistance to lethal challenge with dengue type 4 virus. In each instance the observed resistance appeared to be mediated by E or NS1 antibodies. Thus, naive (unimmunized) mice were rendered resistant to dengue virus challenge by transfer of serum from mice which had previously been: (1) immunized with baculovirus recombinant-expressed E or (2) infected with a vaccinia virus-E recombinant. Passive transfer of serum from mice previously infected with a vaccinia virus-NS1 recombinant also conferred resistance to dengue virus challenge on naive, unimmunized mice. Analysis of the serum of these immunized mice indicated that NS1 induced a high level of NS1-specific antibodies which lysed dengue virus-infected cells in the presence of complement. In contrast, mice immunized with recombinant-expressed E did not develop an antibody response to E detectable by immunoprecipitation and only a very low titer of antibodies could be detected by the neutralization technique. Nonetheless, transfer of sera from vaccinia-dengue E recombinant-infected mice to naive mice, conferred resistance to virus challenge. Since resistance was not conferred by transfer of sera from mice immunized with vaccinia virus it would appear that E specific antibodies were responsible for the observed protective effect.

Deletion analysis of the E glycoprotein was performed with carboxyl deletions which were collinear at their N-terminus. Mice immunized with a vaccinia virus recombinant which expressed only the 60% N terminal amino acids of E induced almost complete resistance to dengue virus challenge, suggesting that a major protective antigenic site (or sites) is located on the N-terminal 60% of the glycoprotein.

Protective efficacy of baculovirus or vaccinia-dengue recombinants in monkeys. Rhesus monkeys were immunized by: (1) infection with a vaccinia recombinant expressing C-M-E-NS1, (2) inoculation with a lysate of moth cells infected with a baculovirus recombinant expressing the same dengue viral proteins or (3) inoculation of a lysate of moth cells infected with a baculovirus recombinant expressing only E. One of 3 monkeys in the second group and one of 3 monkeys in the third group were completely protected from dengue virus challenge as indicated by absence of viremia. Also, one of the other monkeys in the second group was viremic for only a short interval compared to control or unprotected monkeys. These findings suggest that dengue viral E with or without NS1 can induce significant resistance to dengue virus infection in an experimental primate. Immunized monkeys in groups 1 and 2 developed a moderate level of antibodies to NS1, but none of the monkeys in any of the groups developed antibodies to E measurable by immunoprecipitation. However, following challenge with dengue virus, previously immunized monkeys developed a rapid rise of E (groups 1, 2 and 3) and NS1 (groups 1 and 2) antibodies to a higher level than attained by control monkeys suggesting that immunization with recombinant-expressed E or E and NS1, although not completely protective, nonetheless, did prime each immunized monkey for a heightened immune response to these protective antigens. Possible advantage could be made of this priming effect in a 2 stage immunization schedule. Recombinant expressed E and NS1 might be used in the first phase for priming, while infection by an underattenuated dengue mutant might be employed in the second phase to induce a booster response.

Respiratory Viruses

Structure and function of parainfluenza virus genome and gene products. Intracellular synthesis and maturation of parainfluenza virus type 3 (PIV3) hemagglutinin-neuraminidase (HN) surface glycoprotein was analyzed by expression of cDNAs that had been engineered to encode N-terminally truncated HN. Folding of HN occurs to completion in the rough endoplasmic reticulum (ER), however, correct folding alone is not sufficient to assure transport through the exocytotic pathway.

Baculovirus was used to construct recombinants which express PIV3 HN or fusion (F) glycoprotein at a high level for use in structural studies. A new protease cleavage site was introduced near the anchorage domain in the N terminal region of HN or the C-terminal region of F so that these glycoproteins could be easily recovered from virus or infected cells in soluble form for crystallographic analysis.

Antigenic and functional analyses of PIV3 HN and F surface glycoproteins were extended using newly generated monoclonal antibodies (mAbs). For example, a collection of 26 F-specific mAbs was used to define 20 epitopes on F, 14 of which are involved in neutralization of viral infectivity. The 14 neutralization epitopes are distributed among 3 non-overlapping antigenic sites (A, B and C) and one bridge site (AB). Sites AB, B and C are located in close proximity to the fusion site on the mature F molecule. Neutralization-escape mutants selected by neutralizing (N) mAbs were used to identify amino acid residues which are involved in binding of N mAb. None of these sites are in or near the hydrophobic fusion site. Instead, they are located either upstream or downstream of the site suggesting that these distant regions on the linear protein are folded into close proximity to the fusion peptide to create the mature antigenic sites.

Sequence analysis of the respiratory syncytial virus (RSV) genome and gene products. Sequence analysis of the RSV genome is nearing completion. The sequence of the largest gene (6500 nucleotides) which codes for the L protein, thought to be the viral polymerase, has just been completed. Preliminary comparison of the single long open reading of this gene with the

L sequence of other paramyxoviruses has identified two regions of amino acid relatedness which may represent conserved functional domains. Finally, sequence analysis of the 3' leader region which precedes the first RSV gene and the 5' trailer region which is downstream of the most distal gene (L) is almost complete.

RSV fusion (F) glycoprotein. Operational, topologic and functional epitope mapping of the RSV F glycoprotein have been completed. A collection of 18 N mAbs defined 16 neutralization epitopes which are organized into 3 non-overlapping sites (A, B and C) and one bridge site (AB). Fusion-inhibition assays of the N mAbs indicate that sites A, AB and C correspond to domains that affect fusion activity. Consistent with this interpretation is the observation that neutralization escape mutants selected with mAbs binding to site A or AB produce pinpoint plaques. Also, epitopes in sites A, AB or C were found to be less variable among clinical RSV isolates than epitopes on site B.

Immunization of chimpanzees with vaccinia virus recombinants that express the surface glycoproteins of human RSV did not induce effective resistance to RSV infection. The immunogenicity and protective efficacy of recombinant vaccinia viruses that express the two major protective antigens of human RSV, the F and G glycoproteins, were evaluated in chimpanzees. In previous studies in cotton rats and owl monkeys, the F and G glycoproteins expressed by the same recombinants were highly immunogenic and induced a high level of resistance to RSV replication in the lower respiratory tract. In contrast, the recombinants were poorly immunogenic and failed to induced significant resistance in chimpanzees. The vaccinia-RSV recombinants induced a low level of RSV neutralizing antibodies despite the development of large dermal lesions at the site of virus inoculation and the development of high levels of serum antibodies to vaccinia virus. The reasons for the poor immunogenicity and lack of protective efficacy of the recombinant expressed RSV glycoproteins in chimpanzees are not completely clear at this time. However, it is likely that the chimpanzees were not protected against RSV challenge because: (i) it is more difficult to protect a permissive host, such as the chimpanzee, than one which is semi-restrictive, such as the cotton rat or owl monkey and (ii) the limited immune response of chimpanzees to RSV glycoproteins expressed by the vaccinia recombinants was not sufficient to provide effective resistance to RSV in this permissive host. These observations suggest that vaccinia-RSV recombinant viruses would have limited usefulness as vaccines strains for prevention of RSV disease in humans.

Construction and evaluation of adenovirus-RSV glycoprotein recombinants. Using cloned adenoviral DNAs supplied by Wyeth Laboratory, the RSV F gene was placed under the control of an adenoviral promoter in the E3 region and an adenovirus type 5-RSV F recombinant was constructed. The expression of authentic F protein was confirmed by radiolabeling, immunoprecipitation, gel electrophoresis, and cell surface immunofluorescence. This adenovirus-5-RSV recombinant virus was evaluated for immunogenicity and protective efficacy in cotton rats, an animal that is semi-permissive for adenovirus type 5. When administered intranasally, the adenovirus-F recombinant induced a high level of RSV ELISA antibodies, whereas, following administration by the intraduodenal route, the titers of RSV ELISA antibodies were four-fold lower. Surprisingly, however, neither method of immunization with adenovirus-F induced detectable levels of RSV serum neutralizing antibodies, in contrast to the high titers induced by intranasal infection with RSV or intradermal infection with the vaccinia-RSV recombinants. Despite the absence of a detectable neutralizing antibody response, immunization with the adenovirus-F recombinant by either route induced a moderate to high level of resistance to RSV challenge. The high level of resistance to RSV replication observed following intranasal immunization was surprising because of the absence of a significant neutralizing antibody response, suggesting that other aspects of host immunity, such as non-neutralizing antibodies or cellular immunity, might be involved.

Evaluation of cold-adapted mutants of human PIV3 in chimpanzees and volunteers. In collaboration with Dr. Belshe at Marshall University, we have assessed the suitability of cold-adapted (ca) mutants of human PIV3 as attenuated viruses for use as vaccines in humans. In adult volunteers, ca PIV3 (low temperature passage levels 12 and 18) and the wild type parent virus were poorly infectious even at a dose of $10^{7.0}$ $TCID_{50}$. Thus, the attenuating effects of the ca mutations could not be assessed in adults, presumably because repeated prior reinfection by PIV3 had rendered them resistant to infection with wild type PIV3. For this reason evidence for attenuation of the mutants was sought in our closest primate relative, the chimpanzee. Analysis of the ca mutant (12th low temperature passage in cell culture) indicated that it was restricted in replication at least 500-fold in the upper and lower respiratory tract compared to its wild type PIV3 parent. Stability of the ca phenotype and its associated ts phenotype are currently under study. Our observations to date indicate that the ca mutant is attenuated for seronegative chimpanzees and this provides the basis for further evaluation of these promising candidate vaccine viruses in susceptible humans.

Pathogenesis of enhanced pulmonary immunopathology which occurs when cotton rats previously immunized with formalin-inactivated RSV vaccine are subsequently infected with RSV.The pathogenesis of RSV in cotton rats was studied using in situ hybridization to quantitate the number of RSV-infected cells. Infectious virus and infected cells were cleared by one week after the start of infection. We also observed that formalin-inactivated RSV vaccine-enhanced lung histopathology develops despite the fact that effective elimination of infectious virus and infected cells has already occurred suggesting that enhanced pulmonary pathology is the result of: (i) an exaggeration of normal immune mechanisms involved in clearance of virus infection, (ii) aberrant immune responses to infection or (iii) both (i) and (ii).

Immunization of cotton rats with highly purified F glycoprotein in alum induced resistance to RSV challenge demonstrable 6 months after immunization. Unfortunately immunized animals developed an increase in alveolar and bronchiolar pathology during RSV infection which was comparable in extent of potentiation to that induced by formalin-inactivated RSV. This suggests that caution should be exercised in using this type of vaccine formulation for prevention of RSV disease in humans.

Immunotherapy of RSV and PIV3 infection. Intranasal administration of human IgG (with a high titer of RSV neutralizing antibodies) to anesthetized cotton rats at the height of RSV infection results in a rapid, irreversible reduction in pulmonary virus. Application of this form of topical immunotherapy to humans requires the development of a suitable delivery system for IgG. For this reason we investigated the suitability of an ultrasonic nebulizer (particle size <2 microns) for topical administration of human IgG. Cotton rats were exposed to aerosolized IgG at the peak of viral replication (3 days) and then sacrificed one day later. Animals which received 10 minutes of aerosol therapy showed a 6-fold decrease in pulmonary virus. A 15-minute exposure was more effective and resulted in a 30-fold reduction in virus. This form of therapy was more effective than intranasal instillation of droplets of the same preparation of IgG. Inasmuch as current clinical protocols for ribavirin therapy of infants with RSV pneumonia or bronchiolitis require constant exposure to drug aerosol for 3 to 5 days, aerosolized IgG immunotherapy might offer an attractive alternative or adjunct to chemotherapy.

Cotton rats infected with human PIV3 were anesthetized at the time of peak viral replication (day 3), inoculated intranasally with 0.1 ml of convalescent cotton rat PIV3 antiserum, and sacrificed one day later. Undiluted antiserum (reciprocal neutralizing antibody titer = 10,000) reduced infectious virus in the lungs 300-fold. Antiviral activity diminished rapidly with serial 2-fold dilution of antiserum, reaching an insignificant level at dilutions of 1:8 and greater. These observations indicate that PIV3 infection also responds rapidly to topical immunotherapy with human IgG (which usually contains a high concentration of PIV3 neutralizing antibodies). Our observations also suggest that topical immunotherapy may prove to be effective for a variety of viral infections which are limited to the lining mucosa of the respiratory tract.

Influenza A Virus: Evidence for interspecies transfer of avian influenza A virus PB1 polymerase gene to human influenza A virus. Nucleotide sequence analysis of the polymerase genes of the avian influenza A/Mallard/NY/6750/78 virus indicated significant sequence divergence of the avian and human influenza A PB2 and PA genes. In contrast, the PB1 gene of the 1957 human influenza A H2N2 virus was probably derived from an avian influenza A virus by gene reassortment. Putative nuclear localization signals were identified in the PB2, PB1, and PA proteins, while potential active polymerase sites were identified in the PB1 and PA proteins in the form of consensus sequences which are present in other viral RNA-dependent polymerases.

Influenza A Virus: Antibody to a conserved protein, M2, is protective in vivo. M2 is a small viral hydrophobic membrane protein which is conserved among influenza A virus subtypes. mAb to the short extracellular domain of M2 is protective in mice. This is the first evidence that antibody to a conserved influenza virus antigen can provide resistance in vivo.

Influenza A Virus: The B allele of the NS gene of avian influenza A viruses, but not the A allele, attenuates human influenza A virus for squirrel monkeys. The nonstructural (NS) gene of avian influenza A viruses exhibits nucleotide sequence dimorphism. We have referred to these dimorphic forms of the NS gene as allele A and allele B. There is approximately 90% sequence homology within alleles and 72% homology between alleles. All human influenza A viruses analyzed thus far contain an A allele of the NS gene. Single gene avian-human influenza A virus reassortants were constructed which contained an A allele or B allele NS gene from an avian influenza A virus, while all other genes were derived from a human influenza A virus. The resulting reassortants were tested for their ability to grow in the respiratory tract of the squirrel monkey. Reassortants containing a B allele avian influenza NS gene were significantly restricted in growth in the respiratory tract, while reassortants with an A allele NS gene were not. Divergent evolution of the B NS allele in birds probably selected for a gene whose products do not function efficiently in primate respiratory epithelium, especially in cooperative activities involving human influenza A viral proteins.

Influenza A Virus: Evaluation of cold-adapted (ca) reassortant vaccines in the elderly. Evaluation of monovalent and bivalent ca viruses in the elderly indicated that ca vaccines were less immunogenic than inactivated virus vaccine. This suggests that live influenza vaccines do not constitute a suitable alternative to inactivated virus vaccine in this age group. The combined use of both live and inactivated virus vaccine provides only a small increase in mean antibody titer or frequency of a serum or nasal wash response. Thus, little additional benefit would be derived from the combined use of both vaccines. The greatest potential for use of live influenza virus vaccines is probably in infants, children, and young adults.

Influenza B Virus: Influenza B cold-adapted (ca) reassortant viruses are safe and genetically stable in seronegative infants and children. Influenza B/Ann Arbor/1/66 ca mutant is being evaluated as a donor of attenuating genes for wild type (wt) influenza B viruses. Influenza B virus reassortants that derived their HA and NA genes from the wt virus and the remaining six RNA genes from the ca donor virus were shown to be highly infectious, immunogenic, and phenotypically stable in seronegative infants and children. Thus, the ca reassortants derived from this donor virus show promise as candidate live vaccine strains.

Enteric Viral Pathogens

Live attenuated rotavirus vaccine. Rotaviruses are the single most important etiological agents of severe diarrhea in infants and young children in both developed and developing countries. Thus, the need for a rotavirus vaccine is clear. Most of our research effort on a live vaccine has centered on an animal rotavirus, rhesus rotavirus (RRV) which does not appear to be a virus of the human heritage. The basis for the attenuation of this simian rotavirus in susceptible infants and young children appears to be host range restriction which reflects the significant divergence in sequence of RRV genes from the corresponding genes of human rotaviruses. Properties of RRV important to its use as a live vaccine include: (1) failure to produce significant reactions in susceptible young infants, (2) marked restriction in replication in the intestinal tract of infants, (3) satisfactory immunogenicity and induction of resistance to homotypic (serotype 3) rotaviruses and (4) failure to spread to contacts of vaccinees. Recently it was observed that homotypic immunity is necessary for resistance to rotavirus diarrhea in young infants. To meet this need single gene substitution reassortants containing a human rotavirus VP7 (the major serotype-specific neutralization antigen) were prepared with RRV serving as the parent for all other genes in order to provide protection against serotypes 1, 2 and 4; RRV is a serotype 3 virus. These reassortants, now being evaluated in infants, appear to be similar to their RRV parent as regards attenuation and immunogenicity. Fine tuning of vaccine formulation and dosage are now underway. Another approach which utilizes a naturally attenuated rotavirus recovered from asymptomatically infected neonates is also being pursued. Preliminary trials indicate that this candidate strain (M37, serotype 1), is satisfactorily attenuated and immunogenic. Phase II trials are currently underway.

Role of VP4 outer capsid protein in rotavirus virulence. Previous studies in LID suggested that the low virulence of some rotavirus infections in newborn infants was related to specific properties of the infecting strains. Hybridization and sequence analysis of prototype strains isolated from: (i) asymptomatic newborn infants and (ii) infants with diarrhea provided evidence that the fourth gene played a major role in virulence of rotavirus infection of newborns. This gene encodes VP4, an outer capsid neutralization antigen. The VP4 genes of two serotype 1 strains, M37, recovered from a healthy newborn infant ("nursery" strain) and Wa, recovered from an ill infant, were selected to serve as prototypes for the two major alleles of this human rotavirus gene. Additional information bearing on the significance of these VP4 alleles in virulence was sought by analyzing rotavirus strains isolated from: 1) asymptomatic newborns resident in nurseries where rotavirus transmission had persisted for more than one year; 2) sporadic cases of asymptomatic neonatal infection; or 3) older infants with diarrhea. Specific probes that distinguished the Wa and M37 VP4 alleles were used in a dot hybridization assay to classify a collection of rotavirus isolates. Thus far, all rotavirus strains implicated in long term persistence of virus and transmission of asymptomatic infection in a newborn nursery have possessed a VP4 similar to that of the M37 strain. In contrast, Wa-like VP4 was identified in isolates from most ill infants as well as asymptomatic neonates from nurseries where only sporadic infections were detected. These observations suggest that both viral (i.e., VP4) and host factors are involved in the phenomenon of asymptomatic infection in the neonatal period.

Immune response to rotavirus infection and immune correlates of resistance. A recently developed epitope blocking assay (which measures competitive inhibition of binding of mAbs to virus) now makes it possible to measure serotype-specific antibody responses to rotavirus infection. For example, this technique has proven particularly helpful in measuring the antibody response of young infant vaccinees to major protective neutralization epitopes present on rotavirus vaccine strains. The most significant observation to emerge from these analyses was that young infants who had not been infected with any rotavirus in the past responded to immunization with a serotype-specific response, whereas, infants with prior rotavirus experience developed a more broad, heterotypic response. In addition, immunological correlates of resistance were identified when the VP4 and VP7 epitope blocking techniques were used to analyze sera from adult volunteers who were challenged with human rotavirus. Only volunteers who lacked serum antibodies to the VP7-specific epitopes of the challenge strain became ill when virus was fed.

Expression of authentic rotavirus VP4 by a baculovirus recombinant. A complete cDNA of the VP4 gene of porcine rotavirus strain OSU (serotype 4) was inserted into a baculovirus expression vector under the control of its polyhedrin promoter and was expressed in high yield in *Spodoptera frugiperda* (moth) cells. Reactivity of the expressed VP4 with polyclonal and monoclonal antibodies suggested that its neutralization epitopes were antigenically unaltered. Significantly, immunization of guinea pigs with the expressed VP4 product induced antibodies which inhibited hemagglutination of the OSU virus and neutralized its infectivity at high titer. These observations suggest that the VP4 expressed in moth cells maintained its native antigenic structure and thus, represents a source of authentic VP4 suitable for further biophysical and immunological studies. The VP4 of human KU rotavirus (serotype 1) was also expressed in a baculovirus recombinant and preliminary evidence indicates that the expressed VP4 also retains its antigenic properties. Our success in expressing apparently authentic VP4 from two distinct rotaviruses suggests that it should be possible to express the VP4 of a wide variety of rotaviruses in authentic form and use these rotavirus outer capsid VP4 proteins to further define: (1) the extent and distribution of VP4 polymorphism among strains recovered from humans and animals and (2) the role of VP4 in virulence and resistance to rotavirus infection and disease.

Molecular studies of other rotavirus genes and gene products. The nucleotide sequence of first gene segment of the porcine rotavirus Gottfried strain (serotype 4) was determined. This large gene segment encodes the VP1 protein which is present in single-shelled rotavirus particles. It has been suggested that VP1 provides virus transcriptase and replicase enzymatic activities. The VP1 gene was 3302 nucleotides in length, had a single open reading frame capable of encoding a protein of 1088 amino acids and shared 5 consensus sequences with RNA-dependent RNA polymerases of 8 different plant and animal single-stranded RNA viruses. This suggests that VP1 plays a role in mRNA synthesis. In addition, VP3, which is also present in single shell rotavirus particles, exhibits GTP-binding activity suggesting that this protein has mRNA capping enzyme activity.

The sixth gene segment encodes VP6, the major protein of the rotavirus inner capsid. Until now every rotavirus VP6 gene which has been sequenced has contained a unique AUG located 23 nucleotides downstream of its 5' terminus, however, an exception to this rule was recently observed. The VP6 gene of the equine H-2 rotavirus strain contains an additional AUG at residues 132-134, which initiates the translation of a second VP6 protein. Both VP6 products assume a trimeric conformation in the mature virion. The VP6 gene of the H2 strain is the second example of an active second AUG codon in a rotavirus gene. The first example is the VP7 gene as described by others.

Some rotaviruses exhibit an anomalous pattern of migration of genomic RNAs during electrophoresis. This may take the form of an 11th RNA segment which exhibits decreased mobility. Sequence analysis of such an 11th RNA segment derived from a rabbit rotavirus (Alabama strain) indicated that this gene was 1036 base pairs in length, which is 369 nucleotides longer than usual. Two potential non-overlapping open reading frames were identified. It is likely that this unusually large gene was produced by a simple duplication followed by a simple deletion. Genome rearrangements of this type may represent another mechanism for genetic variation among rotaviruses in addition to point mutation and gene reassortment.

HONORS AND AWARDS

Robert M. Chanock

Co-organizer, Cold Spring Harbor Conference on Modern Approaches to Vaccines Including AIDS, September 1988.

Member of the Board of Scientific Governors, Research Institute Scripps Clinic, January 11-12, 1989.

Co-organizer of Fifth Annual Symposium on Animal Virology, Wallace Rowe Symposium, Lister Hill Auditorium, NIH, February 6-7, 1989.

Member of U.S. delegation to Bilateral U.S. National Academy of Sciences and Academy of Sciences of USSR Conference on Biological Weapons Prevention, March 31-April 3, 1989, London, England.

Member of Research Briefing Panel, Institute of Medicine, National Academy of Sciences, on Molecular Biology of Microbial Pathogenesis, Boston, Massachussetts, April 27-28, 1989.

Ad Hoc Reviewer of Antiviral Research Program, US Army Medical Research Institute Infectious Diseases, Fort Detrick, Maryland, March 12-14, 1989.

A.Z. Kapikian

Invited speaker at Fourth Armenian Medical Congress, May 20-22, 1988, Los Angeles, California. Presentation on "Current Developments in Viral Gastroenteritis", May 22, 1988.

Co-chairman of a session on Viral Gastroenteritis at Working Conference on Viral Diseases of the US-Japan Cooperative Medical Science Program, July 19, 1988 in Tokyo, Japan.

Presentation "An update on viral gastroenteritis" at Working Conference (July 18-20, 1988) on Viral Diseases of the US-Japan Cooperative Medical Science Program on July 19, 1988 in Tokyo, Japan.

Co-leader of open discussion portion on research needs at Third NIH Rotavirus Workshop, Bethesda, MD, September 8-9, 1988.

Invited as temporary adviser at 6th meeting of the Scientific Working Group on Immunology, Microbiology and Vaccine Development, 7th Diarrheal Disease Control Program of the World Health Organization, September 27-30, 1988 in Geneva, Switzerland. Dr. Kapikian was a member of the Scientific Working Group.

Invited to be chairman of a session at International Meeting on Molecular Genetics of the Rotaviruses, April 20-21, 1989 at Jouy-en-Josas, France. Chairman of the session on April 20, 1989.

Invited member, Membership Committee of American Epidemiological Society, 1989.

Chairman, Membership Committee, American Epidemiological Society, 1989.

Invited member of Panel on Viral Diseases of US-Japan Cooperative Medical Sciences Program.

Ching-Juh Lai

Invited speaker, University of Massachusetts Medical School, Department of Pharmacology, November 14, 1988. Title: "Use of Subunit Dengue Protective Antigens and Live Recombinant Vaccinia Viruses for Immunization Against Dengue Virus Infection."

Invited speaker, "Modern Approaches to New Vaccines" conference, Cold Spring Harbor, New York, September, 1988. Title: "Use of Dengue Virus Structural Proteins and Nonstructural Protein NS1 Produced by Recombinant Baculovirus for Immunization Against Dengue Virus Infection."

Invited speaker, "WHO Review on the Development of Dengue Vaccines", Vienna, Austria, June 24-25, 1989. Title: "Use of Subunit Proteins and Recombinant Vaccinia Viruses Expressing Nonstructural Protein NS1 for Immunization Against Dengue Virus Infection."

Invited speaker, "2nd International Symposium on Positive Strand RNA Viruses", June 26-30, 1989. Title: "Proper Synthesis of Dengue Virus Nonstructural Glycoprotein NS1 Requires the N-Terminal Hydrophobic Signal Sequence and the Downstream Nonstructural Protein NS2a."

Awarded a research support grant for mapping protective epitopes of dengue virus envelope protein and nonstructural protein NS-1 by the WHO.

Brian R. Murphy

Appointed to the World Health Organization Steering Committee on Respiratory Viruses, 1988.

Editorial Board, Journal of Virology, Jan. 1, 1989 to Dec. 31, 1991.

Invited speaker at Conference on Emerging Viruses: Evolution of viruses and viral diseases. Sponsored by NIAID, Fogarty International Center, the Rockefeller University, May 1-3, 1989, Washington, D.C.

Chairman of Session on Vectors and Vaccines at a Symposium on Respiratory Virus Vaccine Development sponsored by the World Health Organization in Washington, D.C., May 1, 1989.

Robert H. Purcell

Invited speaker, XX Congress of the International Society of Blood Transfusion, London, England, July 10-15, 1988.

Invited speaker, Satellite Symposium on Hepatitis Non-A, Non-B, International Association for the Study of the Liver, Toronto, Canada, November 1, 1988.

Invited speaker, Lecture series on "Molecular Strategies in Vaccine Development", University of Cincinnati College of Medicine, Cincinnati, Ohio, November 15, 1988.

Invited speaker and participant, Workshop on Hepatitis, World Health Organization, London, England, December 1-3, 1988.

Invited speaker, II International symposium on Viral Hepatitis and Hepatocellular Carcinoma, Taipei, Taiwan, December 7-9, 1988.

Invited speaker, International Workshop on Pathogenesis and Prevention of Hepatocellular Carcinoma, Oahu, Hawaii, February 13-15, 1989.

Invited speaker and member of panel, U.S.-Japan Bilateral Science Agreement, Panel on Hepatitis, Gotembe, Japan, March 16-17, 1989.

Invited speaker, Congressional hearings on Non-AIDS Medical Research, Hepatitis Panel, Washington, D.C., April 5, 1989.

Invited speaker, Postgraduate Course: "Frontiers in Gastroenterology and Hepatology", American Gastroenterological Association, Washington, D.C., May 13-14, 1989.

Invited speaker, International Symposium on Molecular Biology of HBV and NANB Virus, Kanazawa, Japan, June 1, 1989.

Michael Bray

Invited speaker, "Modern Approaches to New Vaccines" conference, Cold Spring Harbor, New York, September, 1988. Title: "Mice Immunized with Recombinant Vaccinia Virus Expressing Dengue-4 Structural Proteins and/or Nonstructural Protein NS1 are Protected Against Fatal Dengue Encephalitis."

Kathleen Coelingh

Invited speaker, Negative Strand Virus meeting, Dinard, France, September 5, 1988.

Invited speaker, WHO Symposium on Respiratory Virus Vaccine Development, Washington, D.C., May 1, 1989.

Invited speaker, Plenary lecture at VIth International Conference on Comparative and Applied Virology, Banff, Albert, Canada, October 15-21, 1989.

Peter Collins

Member, Editorial Board of the Journal of Virology, 1989-1991.

Member of International Committee on Taxonomy of Viruses (ICTV).

Suzanne Emerson

Invited speaker, "Modern Approaches to New Vaccines" conference, Cold Spring Harbor, New York, September, 1988. Title: "Identification of the Hepatitis A Virus Genes Involved in Adaptation to Tissue Culture Growth and Attenuation."

Invited speaker, NIH seminar "Genes Involved in *in vitro* Cultivation of Hepatitis A Virus," March 15, 1989.

Barry Falgout

Invited speaker, Dept. of Immunology and Infectious Diseases, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, December 7, 1988. Title: "Expression of Dengue Virus Antigens by Recombinant Vaccinia Virus."

Invited speaker, Dept. of Immunology, Scripps Clinic and Research Foundation, LaJolla, CA, March 13, 1989. Title: "Expression of Dengue Virus Antigens by Recombinant Vaccinia Virus."

Jorge Flores

Invited speaker, Ninth British Society of Gastroenterology Workshop, "Viruses and the gut." Windsor, England, Oct. 2-4, 1988. Title: "Approaches to Rotavirus Vaccination"

Invited speaker, International meeting on Molecular Genetics of the Rotaviruses, Jouy-en-Josas, France, April 20-21, 1989. Title: "Molecular Epidemiology of Human Rotaviruses."

Invited speaker, meeting of rhesus rotavirus vaccine investigators, sponsored by Wyeth-Ayerst, Boulder, Colorado, May 18-19, 1989. Title: "Rhesus Rotavirus Vaccine Development and Field Trials in Venezuela."

Kim Y. Green

Invited speaker, Third NIH Rotavirus Workshop, NIH, Bethesda, MD, Sept. 8-9, 1988. Title: "Use of Monoclonal Antibodies to Detect Serotype-Specific Responses in Rotavirus Vaccinees."

Invited Speaker, American Society for Virology, July 9-13, 1989, London, Ontario, Canada. Title: "An Approach to the Identification of Rotavirus VP4 Serotypes Using Baculovirus-Expressed VP4 Proteins".

Lewis Markoff

Invited speaker, LCI, NIAID, January 1989. Title: "Molecular Biology of Dengue Viruses".

Invited speaker, Johns Hopkins Medical School, Departments of Internal Medicine and Infectious Disease, June, 1989. Topic: "Cross Reactive Antibodies to Clotting Factors Elicited by Flavivirus Infection".

Invited speaker, Department of Viral Biology, USAMRIID, Ft. Detrick, MD, June 1989. Topic: "Cross Reactive Antibodies to Clotting Factors Elicited by Flavivirus Infection".

Roger H. Miller

Invited speaker and moderator at the 25th Conference of the Japanese Association for the Study of Liver Diseases, June 1-2, 1989, Kanazawa, Japan.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00308-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro and In Vivo Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert Purcell, M.D.	Head, HV Section	LID,NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID,NIAID

COOPERATING UNITS *(if any)*

Smith Kline RIT (d'Hondt); FDA (Daemer); Fairfield Hospital, Melbourne, Australia (Gust); AFIP (Ishak); WRAIR (Sjogren)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(INACTIVE 1988-89)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00309-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hepatitis A Virus (HAV)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
Others:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
	Czeslaw Wychowski, Ph.D.	Visit. Assoc.	LID, NIAID
	Edward Cox	Guest Researcher	LID, NIAID
	Patricia Shields	Guest Researcher	LID, NIAID
	Robert Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*
Johns Hopkins Medical School (Karron); Harvard Medical School (Cohen); LIG, NIAID (Maloy); Research Institute of Scripps Clinic, LaJolla, CA (Lerner); Univ. of N.C. (Lemon)

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Hepatitis Viruses Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have produced HAV "epitope libraries" in the expression vector lambda gt 11. A fragment of HAV cDNA representing the entire capsid coding region was isolated. Random fragments of this DNA were produced by limited DNase digestion and these fragments were cloned into lambda gt 11. These libraries were screened by neutralizing monoclonal antibodies, but specific clones were not identified. However, anti-peptide antibodies representing regions from all four capsid proteins readily identified clones. These results suggest that the epitopes recognized by these antibodies are discontinuous or conformational and a different approach will be required to identify them. A larger battery of monoclonal antibodies is now being used to screen the libraries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00311-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Search For New Hepatitis Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Nat'l. Inst. Virol., Pune, India (Arankalle); USUHS, Bethesda, MD (Legters); WRAIR, Wash., DC (Ticehurst, Sjogren); Hosp. for Sick Children, Toronto, Canada (Phillips)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.7	0.3	1.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1980 we described a new form of viral hepatitis caused by a previously unrecognized hepatitis virus with epidemiologic characteristics of HAV (i.e., the virus is fecal-orally transmitted, produces water-borne epidemics and is probably responsible for a significant proportion of endemic hepatitis) but not related to HAV. The disease has been called epidemic or enterically transmitted non-A, non-B hepatitis and hepatitis E (ENANB). Such hepatitis is both epidemic and endemic throughout the Indian subcontinent, central Asia, parts of the Middle East and northern Africa, with probable extension to West Africa. The putative etiologic agent, a 27-30nm nonenveloped virus, was identified in a volunteer study in 1983 and similar virus-like particles have been detected in feces of cases in a number of other outbreaks. The small quantity of such virus-like particles available for study has limited progress.

In 1989 a possible paramyxovirus etiology for neonatal (giant cell) hepatitis was proposed, based on electron micrographic changes seen in the livers of patients with this disease. Collaborative studies, including attempts to transmit the disease to primates, have been initiated.

The objectives of this project are to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A longer term objective is the development of passive and active immunoprophylaxis for these important human pathogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00314-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Woodchuck Virus: Molecular Biological Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roger Miller, Ph.D	Senior Staff Fellow	LID, NIAID
Others:	Cathy Chung, Ph.D.	NRC Fellow	LID, NIAID
	Rosina Girones, Ph.D	Visiting Fellow	LID, NIAID
	Shuichi Kaneko, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HVS	LID, NIAID

COOPERATING UNITS *(if any)*
Div. Molec. Virol. & Immunol., Georgetown U., Wash., D.C. (J. Gerin); New York State College of Veterinary Medicine (B. Tennant).

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TOTAL MAN-YEARS.	PROFESSIONAL	OTHER
4.8	3.8	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. Thus, the WHV/woodchuck system provides a relevant and convenient model for understanding HBV infections of humans. The following experiments were successfully completed: (1) Tests specific for the WHV antigen-antibody systems were developed. (2) Recombinant WHV DNA was transfected into the liver of susceptible woodchucks and acute and chronic WHV infections produced. The complete nucleotide sequence of the infectious recombinant was determined. (3) Mutant WHV genomes were assayed for infectivity in woodchucks and essential genome regions identified. (4) Supercoiled WHV, from the nucleus of infected liver cells, was successfully cloned and the complete nucleotide sequence determined. The sequence of this replicative intermediate is identical to that of the partially double stranded WHV genome found in virions. (5) The complete nucleotide sequence of three additional WHV genomes was determined. It appears that virus subtypes of WHV are evolving, analogous to the surface antigen subtypes of HBV. (6) The mutation rate for WHV replication was found to be $<2 \times 10^{-4}$ changes per genome per year, which is lower than the mutation rate of other viruses that lack polymerases with associated proof-reading functions (e.g. retroviruses and other single stranded RNA viruses). (7) Two transcripts specific for the X gene region of the WHV genome were discovered. One transcript, 0.65 kb in length, was localized to the nucleus of infected liver cells, and found to lack a poly(A) tail at its 3' end. The second transcript, 0.80 kb in length, was shown to be the poly-adenylated version of the 0.65 kb transcript.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00317-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Non-A, Non-B Hepatitis (NANBH) Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

Blood Bank, NIH Clinical Center, Bethesda, MD (Alter, Shih, Esteban); CH, LMG, NIH, Bethesda, MD (Dawid, Sargent). Nihon U. School Med., Tokyo, Japan (Y. Shimizu); Cetus Corp., Emeryville, CA (Sninsky)

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to clone the HCV genome from small quantities of virus, several novel cloning strategies using PCR have been developed. These approaches involve the use of a system of partially random primers, circularization methods if some sequence is known, tailing of RNA and cDNA to produce known ends and combinations of these techniques. These techniques may have broad application to cloning other unknown viral agents.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00370-07 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Andrea Barnes, D.V.M.	Veterinary Medical Officer	LID, NIAID
	Simoy Goldstein, Ph.D.	Expert	LID, NIAID

COOPERATING UNITS *(if any)*

LIG, NIAID, NIH, Bethesda, MD (Fauci, Koenig); Georgetown U. (Formsgard, Gerin, Hirsch, Johnson, London, Olmsted); Delta Primate Center (Murphy-Corb); Soutwest Foundation (Allen)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.7	1.7	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new effort concentrating on animal models of AIDS was undertaken in August, 1987. Two model systems were established: (1) a primate model using the various simian immunodeficiency viruses (SIV), and (2) a feline model using the feline immunodeficiency virus (FIV). These viruses belong to the lentivirus subfamily of retroviruses and induce chronic persistent infection and immunodeficiency in their respective hosts. The SIVs are more or less genetically related to the human immunodeficiency viruses (HIV-1 and HIV-2), while FIV is much less closely related to human or primate viruses based on lack of antigenic cross-reactivity and sequence analysis.

Challenge pools of FIV and several SIVs have been prepared and are undergoing infectivity titration in animals. Disease (simian AIDS) has been produced by SIV and cats have been infected by FIV, but to date no disease has been detected. Both FIV and several SIVs have been cloned and sequenced and their sequences compared with those of other related viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00406-06 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Structure of HAV: Genomic Sequence and Organization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Czeslaw Wychowski, Ph.D.	Visit. Assoc.	LID, NIAID
	Stephen M. Feinstone, Ph.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

RNAs transcribed from the cDNA clones of wild-type or cell culture adapted hepatitis A virus (HAV) or mutants constructed from these clones are being studied to determine the organization of the hepatitis A virus particle. In vitro translation assays are being used to determine gene boundaries. A mutagenesis system was developed to exactly replace entire genes within a cloned DNA. This mutagenesis system was used to construct picornaviral chimeric genomes containing genes from both HAV and poliovirus. Translation of RNAs from these constructs yielded chimeric polyproteins. Processing and assembly properties of these proteins will be studied to determine parameters affecting the structure of the HAV virion and the regions comprising neutralization epitopes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00440-05 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Construction of Full Length Hepatitis A Virus cDNA For Transfection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chimeric cDNAs which contained portions of both wild-type and attenuated hepatitis A virus (HAV) cDNA were constructed. Plus strand RNAs transcribed from constructs were transfected into primate cells to identify those regions of the genome critical for in vitro cultivation. Chimeric viruses harvested from productive transfection experiments were then inoculated into marmosets in order to assess virulence.

Infectivity assays of the chimeric genomes demonstrated that genes within the P2 region determine the ability of the virus to grow efficiently in vitro. Site-specific mutagenesis indicated that two of the genes in this region are both involved. Virulence assays suggest that attenuation for animals and growth in tissue culture are closely coupled. In order to determine if the phenotypes of virulence and in vitro cultivation co-vary or can be dissociated, attempts will be made to grow virulent virus from the wild-type clone by using engineered complementing cell lines or by determining the absolute minimum number of mutations required for growth in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00530-02 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular and Computer Analysis of the Hepatitis B Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Cathy Chung, Ph.D.	NRC Fellow	LID, NIAID
	Rosina Girones, Ph.D.	Visiting Fellow	LID, NIAID
	Shuichi Kaneko, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HVS	LID, NIAID

COOPERATING UNITS (if any)

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Hepatitis Viruses Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to analyze the HBV genome using computer and molecular techniques. Our computer analysis reveals several important findings: (1) We made a detailed study of the organization of the HBV genome and find that it is extremely compact because the virus encodes proteins from overlapping translation frames and because all cis-acting control elements are located within protein encoding sequences. (2) We find that the avian hepadnaviruses possess sequences within the central domain of the core gene that are similar to mammalian hepadnavirus X gene sequences. (3) HBV and retroviruses share features of genome organization and replication. This is significant because it suggests that these diverse virus families evolved from a common ancestral virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00555-01 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Detection of Hepatitis B Virus DNA Using the Polymerase Chain Reaction Assay

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Shuichi Kaneko, M.D.	Visiting Fellow	LID, NIAID
	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HVS	LID, NIAID

COOPERATING UNITS *(if any)*

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Hepatitis Viruses Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In molecular studies, we compared the sensitivity of the polymerase chain reaction (PCR) assay to that of slot blot hybridization for detecting HBV DNA in the serum of patients with chronic hepatitis. In 14 chronic hepatitis patients positive for both HBV surface and e antigens, only 9 were positive for HBV DNA by slot blot hybridization while all 14 were positive by PCR. Also, of 9 patients positive for HBV surface antigen and antibody against HBV e antigen, only 2 were positive for HBV DNA by slot blot analysis while 8 were positive by PCR. Finally, in 8 patients positive for antibody against HBV surface antigen, none were positive for serum HBV DNA by slot blot hybridization but 4 were positive by PCR. We find that analysis by the PCR technique provides a $>10^4$-fold increase in sensitivity over the slot blot hybridization assay. This represents an important breakthrough in sensitivity because it is now possible to detect as few as 3 HBV DNA genomes per sample of serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00323-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Expression of Parainfluenza Type 3 Virus Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.75	0.25	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The intracellular synthesis and maturation of the HN protein of human parainfluenza virus type 3 (PIV3) was investigated by the expression of cDNAs that had been engineered to encode N-terminally truncated HN proteins. These mutants were used to (i) evaluate the possibility of producing a secreted, soluble form of HN that, when expressed by a high-efficiency expression system, could be evaluated as a subunit vaccine and as substrate for structural studies, and (ii) to attempt to identify intermediates in the intracellular pathway of synthesis, folding and oligomerization of HN. The folding of the HN ectodomain into a biologically active and conformationally authentic form occurs to completion in the rough endoplasmic reticulum, however, the finding that two biologically-active HN mutants remained localized primarily in the rough endoplasmic reticulum indicated that correct folding was not the sole prerequisite for movement through the exocytotic pathway. Two of the cDNAs encoded secreted forms of HN, but not in amounts needed for further evaluation.

Studies to evaluate the utility of adenovirus as a recombinant expression vector for PIV3 immunoprophylaxis were initiated with a recombinant adenovirus type 5 virus containing the HN gene inserted in the E3 region. The adenovirus recombinant expressed HN protein that was reactive with PIV3-specific antibodies, however its apparent molecular weight was aberrantly large. Possibility, splicing produced an mRNA that encoded an HN protein fused to an adenovirus protein. If so, an aberrant splice site can be eliminated by site-directed mutagenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00324-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Laboratory Studies of Myxoviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	John T. Treanor, M.D.	Senior Staff Fellow	LID, NIAID
	Mark Perkins, M.D.	Medical Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)
U. of Michigan (Maassab); LVD/NIAID/NIH (Moss, Flexner); St. Judes Children's Research Hospital, Memphis Tenn (Kawaoaka, Webster); Northwestern U., Evanston, IL (Lamb)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antibody to the M2 protein of influenza A virus, a membrane protein conserved among A virus subtypes, is protective in mice. This is the first evidence that antibody to a conserved influenza virus antigen can provide resistance to influenza A virus infection in vivo.

Avian-human influenza A reassortant viruses containing a B allele of an avian influenza NS gene were significantly restricted in growth in the respiratory tract of squirrel monkeys. In contrast, reassortant virus containing an A allele of an avian influenza A virus were not restricted. Divergent evolution of the B allele of the NS gene in birds probably selected for a gene whose products do not function efficiently in cooperative activities involving human influenza A viral proteins.

Nucleotide sequence analysis of the polymerase genes of the avian A/Mallard/NY/6750/78 virus polymerase gene segmnents indicates significant sequence divergence of the avian and human influenza A PB2 and PA genes. In contrast, the PB1 gene of the 1957 human influenza A H2N2 virus was probably derived from an avian influenza A virus by gene reassortment.

A 36-base pair deletion in the NS1 coding sequence of a mutant influenza A virus specified a host-dependent temperature-sensitive (ts) mutation. The ts phenotype was unstable following replication in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00325-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
	John Treanor, M.D.	Senior Staff Fellow	LID, NIAID
	Kathleen Coelingh, Ph.D.	Expert	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
Sema Laboratories, Rockv., MD (J. Philips); Georgetown Univ. (W.T. London); Johns Hopkins U. Schl. Publ. Hlth., Balt., MD (G. Prince); Wyeth Laboratories (P. Hung); Marshall Univ. (R. Belshe)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.8	0.3	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vaccinia-RSV recombinant viruses expressing the fusion (F) or large (G) glycoprotein of RSV were poorly immunogenic in chimpanzees despite efficient replication of the recombinant at the site of intradermal infection. The recombinants induced little resistance to subsequent RSV challenge administered intranasally. Thus, their usefulness for parenteral immunization against RSV in humans would appear to be limited.

Single gene substitution avian-human reassortant viruses bearing the B allele of the NS gene of avian influenza A viruses grew poorly in the upper and lower respiratory tract of primates. It appears that divergent evolution of this gene in birds has selected for an allele which restricts replication of influenza A virus in primates. This NS gene allele could be considered for inclusion in an attenuated influenza A virus vaccine to be used for prevention of disease in humans.

A host range mutant of human adenovirus type 5 and two simian adenoviruses were shown to infect rhesus monkeys by the intranasal route. These observations suggest that these viruses might prove useful as vectors of foreign viral protective antigens which could be tested for immunogenicity and protective efficacy in rhesus monkeys.

A cold adapted mutant of human parainfluenza type 3 virus was attenuated in chimpanzees as indicated by restriction of viral replication in the upper and lower respiratory tract compared to wild type parental virus. An evaluation of the safety and immunogenicity of this candidate vaccine virus can now proceed in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00326-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief, LID	LID, NIAID

COOPERATING UNITS *(if any)*

Flow Labs, Rockv. MD (Potash); Johns Hop. U., Balt. MD (Clements); Vanderbilt U. Sch. Med., Nashville, TN (Wright); Marshall U. Sch. Med., Huntington, WVA (Belshe)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	0.3	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Live attenuated, cold-adapted (ca) influenza A reassortant viruses are less immunogenic than inactivated influenza A virus vaccines in the elderly and thus ca vaccines are unlikely to replace the liscensed inactivated virus vaccine in this population. The greatest potential for use of live influenza virus vaccines is probably in infants, children, and young adults.

A live attenuated ca influenza B reassortant virus derived from the B/Ann Arbor/1/66 ca donor is highly infectious, immunogenic, and phenotypically stable in seronegative infants and children. This suggests that ca recombinants derived from this donor virus have the desired properties for use in a live attenuated viral vaccine.

A clinical trial of attenuated parainfluenza virus type 3 candidate vaccines has been initiated in adults and studies in seropositive children will commence shortly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00327-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Parainfluenza Type 3 Virus Surface Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Kathleen Coelingh, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Susan L. Hall, Ph.D.	IRTA Fellow	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 2.25	PROFESSIONAL: 1.75	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have produced baculovirus recombinants expressing the human parainfluenza type 3 virus (PIV3) hemagglutinin-neuraminidase (HN) protein to extend our previous antigenic and functional characterization of the HN to include structural analysis. The HN protein expressed by initial baculovirus recombinants was shown to be glycosylated, biologically active, immunogenic, and antigenically authentic, but was produced in relatively small quantities. We have now produced a second generation of recombinants which express HN as a fusion product. These new recombinants achieve a level of expression which is 20-fold higher than our initial recombinants. We have also generated recombinants which produce a truncated, secreted form of HN, or a full-length HN containing a genetically-engineered protease cleavage site. We have also produced baculovirus recombinants expressing full-length F protein or F protein containing a new protease cleavage site. The introduction of the cleavage sites into the HN and F will facilitate recovery of soluble expressed PIV3 glycoproteins. Recombinant HN and F proteins will be purified prior to structural analysis.

To extend our antigenic and functional analysis of the PIV3 surface glycoproteins, we have produced monoclonal antibodies (MAbs) to the fusion (F) glycoprotein. These MAbs define 20 epitopes, 14 of which participate in neutralization. The neutralization epitopes are distributed among three non-overlapping antigenic sites (A, B, and C) and one bridge site (AB), and the six non-neutralization epitopes form four distinct antigenic sites. Analysis of the biological activities of MAbs indicated that antigenic sites AB, B, and C correspond to functional domains of the F protein. Antigenic variants have been selected using neutralizing and fusion-inhibiting MAbs, and sequence analysis of these variants has identified specific amino acids which form the neutralization epitopes. These epitopes have been shown to undergo mutation in naturally-occurring clinical isolates which are resistant to MAb-neutralization, suggesting that immune selection plays a role in PIV3 antigenic variation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunity to Paramyxoviruses.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RVS	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory Prince, D.D.S., Ph.D.	NIH Spec. Vol.	LID, NIAID
	Peter Collins, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS (if any)

USUHS, Bethesda, MD (Hemming); Praxis Biologics, Rochester N.Y. (Paradiso); Georgetown University (Jensen).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.4	0.4	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunization of cotton rats with highly purified fusion (F) glycoprotein in alum induced resistance to RSV challenge at 3 and 6 months after immunization. Unfortunately, prior immunization potentiated alveolar and bronchiolar histopathology to a level comparable to that induced by formalin-inactivated RSV. This suggests that caution should be exercised in using this vaccine for prevention of RSV disease in humans.

The suppressive effects of passively administered RSV antibodies on the immune response to RSV F and G glycoproteins expressed by vaccinia-RSV recombinant viruses previously observed were less evident when high doses of purified RSV F and G glycoproteins, i.e., ≥5ug, were used for immuization by the parenteral route. The G protein was more sensitive to immune-suppression than the F glycoprotein.

The pathogenesis of RSV in cotton rats was studied using in situ hybridization to quantitate the number of RSV-infected cells. Infectious virus and infected cells were cleared by one week after the start of infection. Formalin-inactivated RSV vaccine-enhanced lung histopathology develops despite the fact that effective elimination of infectious virus and infected cells has occurred suggesting that enhanced histopathology is the result of: (i) an exaggeration of normal immune mechanisms involved in clearance of virus infection, (ii) aberrant immune responses to infection or (iii) both (i) and (ii).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00368-07 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Analysis of Respiratory Syncytial Virus (RSV) Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	David S. Stec, Ph.D.	NRC Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.95	1.45	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of the difficulty in obtaining sufficient quantities of vRNA for analysis, attempts have been made to synthesize and clone cDNA of the vRNA termini. Attempts to prepare cDNAs by primer extension on intracellular replicative intermediates and readthrough transcripts were not successful, reflecting the low levels of these RNAs in infected cells. Attempts to polyadenylate the 3' end of vRNA and prepare cDNA using oligo dT as primer were unsuccessful, although the reactions worked on control RNA. The use of the polymerase chain reaction to amplify possible low levels of vRNA cDNA was not successful, suggesting that vRNA might not be a possible substrate for polyA polymerase. On the other hand, the 3' end was successfully labeled with pCp added by RNA ligase. Also, synthetic DNA oligonucleotides were successfully added with RNA ligase, and these will be used as a priming site for synthesizing cDNA of the exact 3' (and 5' end) for cloning. This will be done in conjunction with the polymerase chain reaction.

A long-term goal is to produce infectious RSV from a full-length cDNA of vRNA. It is thought, by analogy to other paramyxoviruses, that the minimum structural unit sufficient to initiate productive infection is the nucleocapsid. One possible approach to assembling a nucleocapsid from cDNA would be to coexpress the nucleocapsid components in tissue culture cells using recombinant vaccinia viruses. For example, cells could be coinfected with recombinant vaccinia viruses that express vRNA and the L, N, P and possible other RSV proteins. Alternatively, infectious nucleocapsids could be assembled in vitro by coupled transcription and translation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00372-07 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

RSV Proteins: Roles in Host Immunity and Immunoprophylaxis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LVD, NIAID, NIH (Moss, Buller); Johns Hopkins U. Sch. of Pub. Hlth, Balt., MD (Prince); Wyeth Laboratory, Rancor, PA (Davis, Hung).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.75	0.25	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating approaches for utilizing recombinant DNA techniques in developing a vaccine for human respiratory syncytial virus (RSV). Previously, vaccinia virus recombinants that express individually the RSV F and G glycoproteins were shown to be highly immunogenic and effective in inducing resistance to RSV infection in rodents and non-human primates. The same recombinants were evaluated in chimpanzees, an animal model that is highly permissive for RSV replication. Surprisingly, intradermal inoculation induced only moderate levels of RSV-specific serum antibodies. And, upon intranasal RSV challenge, only a marginal reduction was observed in RSV replication in the respiratory tract. Thus the existing vaccinia viral vectors do not appear to be appropriate for immunoprophylaxis against RSV. However, they represent valuable laboratory reagents for investigating the roles of individual proteins in host immunity and viral pathogenesis in experimental animals. To that end, vaccinia virus recombinants have been constructed with seven other RSV genes, namely the 1C, 1B, N., P, M, 22K and 1A genes.

The use of adenovirus as a viral vector offers several potential advantages for RSV immunoprophylaxis. An adenovirus type 5 recombinant that expresses the RSV F protein was constructed, authentic expression was confirmed, and the recombinant was evaluated for immunogenicity and protective efficacy in cotton rats. When administered intranasally, the adenovirus-F recombinant induced high levels of RSV-specific serum antibodies and nearly-complete resistance to RSV infection. Administration by the intraduodenal route induced moderate levels of RSV-specific antibodies and moderate levels of resistence to RSV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00457-04 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Immune Response to Paramyxovirus Infection or Vaccination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Gregory Prince, D.D.S., Ph.D.	Spec. Volunteer	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

U. Rochester Sch. Med., Rochester, N.Y. (Walsh); Children's Hosp. Nat. Med. Ctr., D.C. (Kim, Parrott); U. North Carolina, Chapel Hill, N.C. (Henderson)

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Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1988-89

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00497-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of the Fusion Glycoprotein of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Kathleen Coelingh, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS *(if any)*

Food and Drug Administration (J. A. Beeler, M.D.)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used 18 neutralizing monoclonal antibodies (MAbs) specific for the respiratory syncytial virus (RSV) fusion (F) glycoprotein to construct operational, topologic, and functional epitope maps of this F glycoprotein. The MAbs define 16 neutralization epitopes which are organized into three non-overlapping antigenic sites (A,B,and C) and one bridge site (AB). Neutralization and fusion-inhibition tests of the MAbs indicated that antigenic sites A,AB,and C correspond to domains of the F protein that affect syncytium-formation, whereas antigenic site B is more distant from the fusion site. This interpretation is supported by two additional observations. First, MAb-resistant variants selected with site B MAbs produce normal plaques, whereas those selected with MAbs binding epitopes in sites A and AB produce pinpoint plaques. Second, epitopes in antigenic sites A,AB,and C (which correspond to domains close to the fusion site) were less variable among RSV clinical isolates than were epitopes in antigenic site B. Sequence analysis of the F genes of antibody-resistant mutants is currently in progress. This information will allow us to identify amino acid residues which are involved in binding neutralizing antibodies and which directly affect syncytium-formation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00498-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis, Processing and Functions of the Proteins of Human RSV

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	David S. Stec, Ph.D.	NRC Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.15	.65	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of the respiratory syncytial virus (RSV) mRNAs by cDNA cloning, sequencing and related techniques has identified ten different mRNAs, and to date a single encoded polypeptide has been identified for each. We are interested in (i) continuing the analysis of the viral mRNAs and their translational open reading frames (ORFs) to detect possible additional mRNAs and polypeptides and to learn more about the details of RSV gene expression, (ii) defining the extent of sequence diversity among RSV isolates, and (iii) analyzing the individual RSV proteins to determine their intracellular and virion locations and their functions. We have continued the analysis of the 1A protein (whose name has been changed to SH [small hydrophobic] to be consistent with the nomenclature for simian virus 5 and mumps virus). The SH protein was shown to be an integral membrane protein that is processed by a complex intracellular pathway that results in two nonglycosylated and two glycosylated species. The processing pathway and structural differences between the different forms have been shown to be conserved between the two RSV antigenic subgroups, have been partially defined, and are under further investigation using site-specific antibodies, recombinant expression vectors and site-directed mutagenesis. Three of the four forms of SH were expressed at the cell surface but, in a preliminary evaluation, immunization of cotton rats with a recombinant vaccinia virus that expresses SH did not induce significant protective immunity, suggesting that SH is not an important protective antigen despite its role as an RSV surface glycoprotein. Other experiments to characterize the RSV gene products are described.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00458-04 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Genetic Variations Among Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS 0	PROFESSIONAL 0	OTHER 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

INACTIVE 1988/89

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00459-05 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Dengue Virus Proteins Using Baculovirus as a Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Robert Chanock, M.D.	Chief	LID, NIAID
	Ruhe Men, M.D.	Special Volunteer	LID, NIAID
	Michelle Pethel	Microbiologist	LID, NIAID
	Yi-Ming Zhang, M.D.	Visiting Assoc.	LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, D.C. (Summers, Eckels, Dubois); Rochester General Hospital, Univ. of Roch., Rochester, N.Y. (J.J. Schlesinger)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have employed the high yielding baculovirus-insect cell system to achieve expression of dengue viral proteins from cloned DNA sequences. A recombinant baculovirus containing dengue type 4 virus cDNA that codes for the three structural proteins, i.e., capsid (C) protein, membrane (M) protein, and envelope (E) glycoprotein and nonstructural proteins NS1 and NS2a was constructed. Infection of cultured insect cells with this recombinant virus produced what appeared to be authentic E and NS1. Immunization of mice with these dengue protein products induced complete resistance to fatal dengue virus encephalitis. Analysis of seroresponse showed that mice immunized with a recombinant virus-infected cell lysate developed a high level of antibodies specific to NS1, whereas antibodies to E or PreM were either low or not detected. In an effort to elucidate this protective effect, recombinant baculovirus that expressed only E was constructed utilizing the N-terminal 71 amino acids of the respiratory syncytial virus G glycoprotein as a signal for processing of the dengue envelope glycoprotein. Mice immunized with a lysate of cells infected with this recombinant virus failed to develop a detectable seroresponse to E, nonetheless these mice were completely resistant to dengue virus challenge. Our observations suggest that the E and/or NS1 glycoproteins of dengue expressed by baculovirus recombinants may prove useful in immunoprophylaxis of human disease caused by dengue viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00475-03 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Complete Nucleotide Sequence of Dengue Type 4 Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ching-Juh Lai, Ph.D	Head, MVB	LID, NIAID
Others:	Bangti Zhao, Ph.D	Visit. Scientist	LID, NIAID
	Lewis Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1988-1989

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00476-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Engineering the Genome of Dengue Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS 0	PROFESSIONAL 0	OTHER 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1988-1989

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00477-04 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Dengue Envelope Protein Sequences by Recombinant Vaccinia Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVBS	LID, NIAID
Others:	Ruhe Men, M.D.	Special Volunteer	LID, NIAID
	Michael Bray, M.D.	Sr. Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

WRAIR, Washington, D.C. (Eckels)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We prepared DNA sequences coding for the complete dengue type 4 virus E glycoprotein and used this clone to construct a series of carboxyl deletions which were collinear at their N terminus. Vaccinia recombinant viruses which expressed these DNA sequences produced the predicted dengue E products ranging in size from 50-100% of E. Each of these E products were glycosylated as indicated by their sensitivity to endoglycosidase F digestion. Subsequently the recombinants were tested for their ability to induce protective immunity in mice against lethal challenge with dengue virus. Mice immunized with V (E 99%) were almost completely protected (mortality 11%). On the other hand, mice immunized with V (E 90%), V (E 70%), or V (E 66%) had a mortality rate of 62%, 70%, or 50% which did not differ significantly from the mortality rate of 70% in the control group. Interestingly, mice immunized with V (E 60%) were almost completely protected (mortality rate 10%). These results, if confirmed, would suggest that one or more of the protective epitopes are located within the N-terminal 60% region of the E glycoprotein. The DNA sequences coding for the full-length E are being expressed using the baculovirus-insect cell system and protective immunity of this complete glycoprotein will be evaluated in mice and in monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00499-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Selected Dengue Structural Protein Sequences by Recombinant Vaccinia Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Lewis Markoff, M.D.	Medical Officer	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LVD, NIAID, NIH (B. Moss)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have constructed recombinant vaccinia viruses expressing dengue virus proteins from cloned DNA for use in experimental immunoprophylaxis. Recombinant vaccinia virus containing the capsid (C), pre-membrane (pre-M) and envelope (E) glycoprotein genes of strain 814669 dengue 4 virus expresses pre-M and E glycoproteins. Mice immunized with this recombinant were protected against an intracerebral challenge of 100 LD50 of strain H241 dengue 4 virus. Vaccinia recombinants expressing only E also protected mice against dengue virus encephalitis. Serum obtained from mice following immunization with an E recombinant contained a low level of dengue virus neutralizing activity, and resistance to challenge was conferred by transfer of serum, indicating that humoral immunity plays a role in protection. A recombinant which contains the C and pre-M genes produces apparently authentic pre-M. Mice immunized with this recombinant were partially protected against challenge, indicating that C and/or pre-M may also be recognized as protective antigens and contribute to immunity. A recombinant which expresses pre-M and E of the S-1 candidate vaccine strain of dengue 2 virus induced solid protection against challenge with 100 LD50 of New Guinea C strain dengue 2. Mice immunized with the dengue 2 recombinant were cross-protected against dengue 4, but animals immunized with the dengue 4 C-pre-M-E recombinant did not show resistance to dengue 2. Neither recombinant protected against dengue 1. Rhesus monkeys were immunized with a vaccinia recombinant expressing dengue 4 pre-M and E and non-structural protein NS1. The monkeys developed antibodies to NS1, but were not protected against homotypic challenge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00500-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Processing and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NSI

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barry Falgout, Ph.D.	IRTA	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Michael Bray, M.D.	Sr. Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

Rochester General Hospital (Schlesinger)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nonstructural protein 1 (NS1) of dengue virus induces protective immunity in mice. We studied the processing and immunogenicity of NS1 expressed by recombinant vaccinia virus. We constructed one series of recombinants that express NS1 in combination with other dengue proteins, and a second series that express subfragments of NS1. Studies of expression of NS1 demonstrated that the downstream NS2a sequence was required in cis for cleavage of the NS1/NS2a junction. Kinetic studies revealed that these constructs initially synthesize NS1 as a precursor containing NS1 plus NS2a. The precursor is rapidly cleaved to produce NS1. Analysis of mutants containing deletions from the C-terminus of NS2a show that the last 20 residues of NS2a are dispensable for this cleavage. Shortening of NS2a between 42 and 65 amino acids results in precursors which are more slowly cleaved, while deletions of 68 or more residues completely abrogate cleavage. Mice immunized with a recombinant expressing authentic dengue virus type 4 (DEN4) NS1 survived lethal intracerebral DEN4 challenge although most of these animals exhibited mild encephalitic symptoms. In contrast, only 35% of animals immunized with a recombinant expressing authentic dengue virus type 2 (DEN2) NS1 survived DEN2 challenge, and none survived DEN 4 challenge. Viruses expressing subfragments of DEN4 NS1 did not protect mice. The level of serum antibody to NS1 in different groups of vaccinated animals, as measured by RIPA, ELISA, and complement-dependent cytolysis, exhibited a direct correlation with the extent of protection and the authenticity of the NS1 product. Passive transfer of serum from immunized animals protected unimmunized animals from lethal DEN4 challenge. We conclude that antibody to NS1 can protect mice against dengue virus encephalitis, and that complement-dependent lysis of infected cells may be an important component of that protection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI00501-03 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of the Processing of Dengue Viral Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Processing of the dengue virus pre-membrane (pM) protein was studied during cell-free translation of RNA transcripts prepared from cloned dengue DNA containing capsid, pM, and N-terminal envelope glycoprotein (E) sequences. To elucidate the mechanism for processing, strategic mutations were produced in capsid and pM sequences by site-directed mutagenesis of dengue DNA, and mutant RNA transcripts were then translated. Cleavage at the capsid-pM and pM-E sites is mediated by signal peptidase. Such signal-directed cleavage function appears to be linked to the translocation event during protein synthesis. This function is served by a hydrophobic segment (amino acid residues 100-113 in the dengue precursor) that occurs at the capsid-pM junction. Recognition of the "signal" does not require prior cleavage of N-terminal capsid sequences. Additional mutations were made in nucleotide sequences encoding the C-terminal hydrophobic segment of pM, thought to contain the pM transmembrane and the E signal sequences in tandem separated by Arg. In separate mutant DNAs, segments encoding each hydrophobic segment and the interrupting Arg have been deleted. These mutations have been cloned into a larger fragment of dengue DNA representing capsid, pM, E, and N-terminal NS-1 sequences. Determination of phenotype in cell-free and in vivo systems is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00502-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigenic Analysis of the Dengue Virus Envelope Glycoprotein (E) Using Synthetic Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Laraine Henchal	Microbiologist	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVBS	LID, NIAID

COOPERATING UNITS *(if any)*

Research Inst. of Scripps Clinic, La Jolla, CA (R. Houghton)

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Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thirty-eight synthetic peptides have been used to identify potential linear B-cell epitopes in the dengue 4 envelope glycoprotein (E) sequence. Available information shows that the immune response to peptides or larger polypeptide fragments of protein antigens may be enhanced if such fragments contain T helper cell epitopes, in addition to those recognized by B cells in the humoral immune response. To identify T helper cell epitopes in the dengue 4 E sequence, the sequence was subjected to a computer analysis designed to predict segments expected to assume an amphipathic α-helical configuration in the native protein, since a significant proportion of identified T cell epitopes may assume such a shape. Two segments were identified by this analysis, residues 396-408 and 423-447 in the 493 amino acid sequence. The 38 synthetic peptides administered as 13 groups of three peptides each were then used to prime the E immune response to a vaccinia recombinant expressing all the structural proteins and NS1 in Balb/c ($H\text{-}2^d$) and CBA/N ($H\text{-}2^k$) mice. In this manner, six segments of the E sequence have been identified which may contain T helper cell epitopes for Balb/c mice. These include segments defined by residues 76-126, 157-210, 268-301, 330-368, 367-406, and 405-433. Future plans include analysis of results using CBA/N mice and fine definition of suspected sites by a repeat of the experiment using individual peptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00503-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Expression of Dengue Virus Antigens Using A Synthetic Signal Peptide.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1988-89
(Merged with Z01 AI 00499-03)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00531-02 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Functional Analysis of Dengue Nonstructural Proteins, NS3 and NS5

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Barry Falgout, Ph.D.	IRTA	LID,NIAID
	Yi-Ming Zhang, M.D.	Visiting Assoc.	LID, NIAID
	Annie Cahour, Ph.D.	Visiting Associate	LID, NIAID
	Thomas McCarty	Microbiologist	LID, NIAID

COOPERATING UNITS *(if any)*

Infectious Disease Unit, University of Massachusetts Medical School, Worcester, MA
(F. Ennis, I. Kurane, and A. Rothman)

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Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

We sought to investigate the possible role that sequences upstream and downstream of NS3 might play during processing of NS3 from the dengue viral polyprotein. Recombinant vaccinia viruses, V (NS3- NS4a) and V (NS3-NS4a-NS4B), containing downstream sequences were constructed. Cells infected with these recombinants produced the respective NS3-NS4a, and NS3-NS4a-NS4b fusion proteins. On the other hand, recombinant viruses containing upstream nonstructural protein sequences, V (NS1-NS2a-NS2b-NS3) and V (NS2a-NS2b-NS3) produced what appeared to be authentic NS3 suggesting that cleavage at the NS3 N-terminus had occurred and that NS2a-NS2b was responsible for the cleavage. To facilitate further analysis of NS3 processing, recombinants V (NS1-NS2a-NS2b-NS3-NS4a-NS4b) and V (NS2a-NS2b-NS3-NS4a-NS4b) containing both sets of upstream and downstream sequences were constructed. Analysis of lysates of cells infected with these recombinants showed that a protein band similar in size to authentic NS3 had been produced indicating that both termini of NS3 had been properly cleaved from the precursor polyprotein. These results suggested that NS2a-NS2b is required for proper cleavage at the NS2b-NS3 and NS3-NS4a junctions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00556-01 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epitopes on Dengue Virus Nonstructural Protein NS1 That Mediate Protection Against Dengue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroyuki Hori, M.D.	Visiting Associate	LID, NIAID
Others:	Barry Falgout, Ph.D.	IRTA	LID, NIAID
	Michael Bray, M.D.	Sr. Staff Fellow	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

Rochester General Hospital, University of Rochester, Rochester, NY (J.J. Schlesinger)

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SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vaccination of mice with: (i) subunit dengue NS1 expressed by a baculovirus recombinant or (ii) a live recombinant vaccinia virus expressing NS1 provides protection against lethal challenge by dengue virus. The mechanism responsible for protection is thought to be mediated by cytolysis of infected cells by NS1 antibodies since a small fraction of NS1 is displayed on the surface of infected cells. We sought to identify the epitopes on the NS1 molecule that are involved in eliciting an antibody response and that provides protection. Initially, we constructed a recombinant vaccinia virus containing the complete NS1 coding sequence V (NS1 100%). Infection of cells with this recombinant produced apparently authentic NS1 at a level 2-3 fold higher than that produced by recombinant V (NS1-NS2a) or V (C-M-E-NS1-NS2a) from which NS1 was cleaved from the dengue polyprotein by downstream NS2a. A series of vaccinia viruses expressing overlapping or non-overlapping fragments of NS1 were also constructed. Interestingly, the amount of NS1 protein detected in cells infected by these mutants was greatly reduced as compared to that of the full-length NS1 expressed by V (NS1 100%). It remains to be determined whether greater stability of the full length NS1 product or its greater binding affinity for NS1 antibodies is responsible for these observations. In any case, it is clear that the C terminal 22 amino acids of NS1 must be present for efficient expression of an immunoprecipitable NS1 product. Several of these vaccinia recombinants are being tested in mice for ability to induce protective immunity against dengue virus disease. Current plans involve analysis of seroresponse by ELISA and by complement-dependent cytolytic antibody assay.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00557-01 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-reactive Antibodies to Clotting Factors in Patients Infected with Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Laraine S. Henchal	Biologist	LID, NIAID

COOPERATING UNITS *(if any)*

WRAIR, Bangkok, Thailand (B.L. Innis); Scripps Institute, La Jolla, California (R. Houghten)

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Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Computer analysis of the predicted amino acid sequence of the dengue envelope glycoprotein (serotype 4, strain 814669) revealed that it contains a sequence of 20 amino acids (residues 101 to 121) with similarity to a 21 amino acid sequence contained in a family of serine proteases which function in blood clotting (plasminogen, prothrombin, Factor X, tissue plasminogen activator, urokinase). The dengue envelope sequence in question is highly conserved among flaviviruses. ELISA using murine hyperimmune ascitic fluids indicated that dengue but not Japanese encephalitis (JE) or St. Louis encephalitis virus antibodies could cross-react with plasminogen and prothrombin. ELISA using peptides confirmed the site-specificity of the cross-reactivity with plasminogen. ELISA using human sera from Thai patients revealed that about 70% of individuals experiencing a secondary dengue infection developed a transient cross-reactive antibody response to plasminogen (IgG class antibody detected in acute but not convalescent sera). In contrast, sera from ten patients infected with JE virus were negative compared to a panel of 17 controls. Cross-reactive antibodies are predicted to bind plasminogen 19 amino acids downstream from the serine active site. Further studies are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00333-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: <1	PROFESSIONAL: <.1	OTHER: <.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses have been studied extensively in many parts of the world predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness in infants and young children. There has been a paucity of longitudinal viral gastroenteritis studies that yield not only important "denominator" data but also valuable insights into the natural history of a pathogen or illness, with special emphasis on epidemiologic, immunologic and laboratory data. We, therefore, initiated an intensive examination of anal swab and serum specimens obtained during a previous LID long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless, but otherwise normal children. Anal swabs and blood specimens were obtained routinely from the youngest age groups, which ranged from 6 months to about 5 years of age during various periods of the study. Surveillance was carried out by a trained medical staff. Children were housed in cottages according to their age. Rectal temperatures were obtained on each child daily; routinely, a physician examined any child with a rectal temperature of 100.6°F or greater. Careful medical records were kept by the nursing staff on all children. Thus, with this background we plan to investigate the natural history of rotavirus infections in a longitudinal manner employing newly developed techniques such as serotyping of rotaviruses with VP7 specific monoclonal antibodies and determining the epitope-specific serologic response in sequential sera to determine the scope of homotypic and heterotypic responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00338-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning of Rotavirus Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visit. Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1988-89

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00339-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation and Serotypic Characterization of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID, NIAID
Others:	Mitzi Sereno	Microbiologist	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

Moredun Research Institute, United Kingdom (Snodgrass); Ohio Agricultural Research and Development Center, Ohio (Theil); Texas A & M Univ. (Woode)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	<1/12	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bovine rotavirus strain 678, which was originally isolated from a diarrheic calf in Great Britain, was found to belong to a newly established serotype 8 (prototype strain HRV 69M) by plaque reduction neutralization (PRN) assay. In addition, this relationship has now been confirmed by sequence analysis. Bovine rotavirus strain ID, which was a "supershort" electropherotype, was found to belong to serotype 6 (prototype bovine rotavirus NCDV strain which has a "long" electropherotype) by PRN assay. Bovine rotavirus strain B223 was shown, by a one-way cross-neutralization assay, to be distinct from any of the 9 established serotypes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00340-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Experimental Studies of Various Rotaviruses and Their Reassortants in Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Mitzi M. Sereno	Microbiologist	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

Ohio Agricultural Research and Development Center, Ohio State Univ., Wooster, Ohio (Saif).

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	<1/12	.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Continued efforts have been made to delineate the genetic mechanisms underlying pathogenicity of rotavirus in the newborn gnotobiotic piglet model.

In these studies the VP4 and VP7 genes have been shown to play an important independent role in rotavirus growth in the gut and in induction of diarrhea. However it appears that in addition to these two outer capsid-encoding genes one or additional genes are required for the induction of diarrhea. Elucidation of the constellation of genes required for rotavirus virulence may have important implications for the development of effective rotavirus vaccine strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00341-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Live Attenuated Rotavirus Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)* U. Rochester, NY (Dolin); Johns Hopkins U., (Black, Clements, Midthun); U. Md. (Levine); U. Umea, Sweden (Gothefors); (U. Tampere, Finland (Vesikari); Vanderbilt U. (Wright); Marshall U. (Belshe); Dept. Med. Res., Min. Health, Rangoon, Burma (Moe) Instituto de Biomedicina, Caracas, Venezuela (Perez-Schael)

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Epidemiology Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.5	.5	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses are the single most important etiological agents of severe diarrhea in infants and young children in developed and developing countries. Thus, the need for a rotavirus vaccine is clear. Most of our research effort on a live vaccine has centered on an animal rotavirus, rhesus rotavirus (RRV) which does not appear to be a virus of the human heritage. The basis for the attenuation of this simian rotavirus in susceptible infants and young children appears to be host range restriction which reflects the significant divergence in sequence of RRV genes from the corresponding genes of human rotaviruses. Properties of RRV important to its use as a live vaccine include: (1) failure to produce significant reactions in susceptible young infants, (2) marked restriction in replication in the intestinal tract of infants, (3) satisfactory immunogenicity and induction of resistance to homotypic (serotype 3) rotaviruses and (4) failure to spread to contacts of vaccinees. Recently it was observed that homotypic immunity is necessary for resistance to rotavirus diarrhea in young infants. To meet this need single gene (VP7) substitution reassortants were prepared with RRV in order to provide coverage against serotypes 1, 2 and 4; RRV is a serotype 3 virus. These reassortants, now being evaluated in infants, appear to be similar to their RRV parent as regards attenuation and immunogenicity. Fine tuning of vaccine formulation and dosage are now underway. Another approach involving a naturally attenuated rotavirus recovered from asymptomatically infected neonates is also being pursued. Preliminary trials indicate that this candidate strain (M37, serotype 1), is satisfactorily attenuated and immunogenic. Phase II trials are currently underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00342-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Albert Z. Kapikian, M.D. Head, Epidemiol. Sect. LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The electron microscope has been a mainstay in the study of gastroenteritis viruses. Two major groups of gastroenteritis viruses - the 27nm Norwalk virus and the 70nm human rotaviruses - were discovered at NIH and in Australia, respectively, (as well as the hepatitis A virus at NIH, in collaboration with the Hepatitis Section) with the use of the electron microscope. It is remarkable that in this era of tissue culture virology, these agents were discovered initially without the use of an in vitro tissue culture system, since they could not be grown directly from clinical specimens in cell culture. We have applied the term, "direct virology," to this method of examining viruses from clinical specimens directly by EM.

Although second and third generation tests have been developed for the detection of the Norwalk group and the rotaviruses, the electron microscope is still an indispensable tool for the study of these gastroenteritis viruses. It is also the most rapid diagnostic tool for detection of rotavirus from a clinical specimen and is the only method available for diagnosing infection with certain 27nm viruses associated with epidemic nonbacterial gastroenteritis. It also is important for: (i) providing direct visualization of virus particles from density gradients (to establish their morphologic appearance, e.g., single or double capsid, integrity of capsid structure, and to determine presence or absence of particles or their quantitation), (ii) visualizing the site of activity of monoclonal antibodies, and (iii) serologic studies performed by immune electron microscopy. However, its most important and creative role is in its application to the detection of new, heretofore unknown, agents of acute infectious gastroenteritis and other diseases as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00343-08 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Sec.	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<1/12	<1/12	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Norwalk virus is one of a group of small, noncultivatable, 27nm viruses which are important etiologic agents of epidemic gastroenteritis in older children and adults. The adaptation of biotin-avidin immunoassays for detection of Norwalk virus antigen and for detection of seroresponses to the virus allowed us to examine several outbreaks of nonbacterial gastroenterititis at various locations including employees at Children's Hospital, Washington DC, U.S. Navy personnel aboard a Navy vessel in South America and individuals at the U.S. Soldiers' and Airmen's Home in Washington DC. The Norwalk virus itself did not appear to be the etiologic agent of any of the outbreaks although on the basis of high levels of Norwalk virus antibody in acute phase serum in some individuals in the Children's Hospital study it is probable that a Norwalk-related virus was associated with at least some of the cases at that location. Attempts to cultivate Norwalk virus in tissue were continued and thus far have not been successful.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI00346-08 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Genetic Characterization of the Rotavirus Fourth Gene by Sequencing and Hybridization Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Kapikian, M.D.	Head, Epidemiol. Section	LID, NIAID
	Robert Chanock, M.D.	Chief, LID	LID, NIAID

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies at LID suggested that the low virulence of some rotavirus infections in newborn infants was related to specific properties of the infecting strains. Hybridization and sequence analysis of prototype strains isolated from: (i) asymptomatic newborn infants and (ii) infants with diarrhea provided evidence that the fourth gene which encodes VP4 played a major role in rotavirus virulence and played an important role in the outcome of infection in newborns. Two prototype serotype 1 strains, M37, recovered from a healthy newborn infant ("nursery" strain) and Wa, recovered from an ill infant, were used to represent two major alleles of the fourth gene which encodes VP4, an outercapsid neutralization protein.

Additional information concerning the significance of these VP4 alleles in virulence was sought by analyzing rotavirus strains isolated from: 1) asymptomatic newborns resident in nurseries where rotavirus transmission had persisted for more than one year; 2) sporadic cases of asymptomatic neonatal infection; or 3) older infants with diarrhea. Specific probes that distinguished genes related to the fourth gene of Wa or M37 were used in a dot hybridization assay to classify the field strains. Our observations indicate that persistent asymptomatic rotavirus transmission in a newborn nursery was always caused by strains with a VP4 similar to that of the M37 strain. In contrast, Wa-like VP4 was identified in isolates from most ill infants as well as asymptomatic neonates from nurseries where only sporadic infections were detected. These observations suggest that both viral and host factors are involved in the phenomenon of asymptomatic infection in the neonatal period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI00446-05 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Testing of Rotavirus Vaccine Candidates in Venezuela

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID

COOPERATING UNITS *(if any)*

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Epidemiology Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	.4	1.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An effective rotavirus vaccine should have a significant impact on the survival of hundreds of thousands of infants and young children in the developing countries since rotaviruses are the most important agents of severe dehydrating diarrhea in this age group throughout the world. Because of the variable efficacy provided by live monovalent rotavirus vaccines containing an animal rotavirus strain, it has become apparent that homotypic immunity against each of the four most common rotavirus serotypes which circulate in humans may be required in order to induce broad protection. This requirement for a polyvalent vaccine has led us to evaluate the rhesus rotavirus vaccine (RRV vaccine) in combination with reassortants, derived from this strain, representing serotype 1, 2 or 4. RRV is serotype 3. Several phase 1 studies designed to study the antigenicity and immunogenicity of different formulations of this quadrivalent vaccine have been performed. Although the antigenicity of this combination is not uniform against all four serotypes, the data obtained thus far justify its continued testing in efficacy trials which are in progress.

We have also evaluated the naturally attenuated M37 strain of serotype 1 rotavirus (a human strain originally isolated from an asymptomatic newborn infant) as a rotavirus vaccine candidate. Experience of other scientists indicates that neonatal infection with human rotaviruses induces resistance against severe rotavirus diarrhea later in life. The M37 strain has proven to be safe and immunogenic in young infants, thus warranting further evaluation in clinical efficacy trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI00450-05 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Relationship of Outer Capsid Protein VP4 of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visit. Assoc.	LID, NIAID
Others:	Kazuo Nishikawa, M.D.	Visit. Assoc.	LID, NIAID
	Koki Taniguchi, Ph.D.	Visit. Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

By primer extension, we determined the complete nucleotide sequence of the gene encoding the outer capsid protein VP4 of human rotavirus strain K8. The VP4 gene is 2,359 base pairs in length with 5'- and 3'-noncoding regions of 9 and 25 nucleotides, respectively. The gene contains a long open reading frame of 2,325 bases capable of coding for a protein of 775 amino acids. When compared to other human rotaviruses, the VP4 of the strain K8 had an insertion of one amino acid after the 135th residue as found in simian rotavirus strains, and, in addition, it had a deletion of one amino acid (575th residue). The VP4 gene of K8 was only 60-70% homologous in amino acid sequence to the VP4 of other virulent human rotaviruses. This is an unusual observation since the VP4s of all other virulent human rotavirus strains exhibit greater than 90% amino acid homology. In contrast, the VP7 amino acid sequence of the K8 strain was quite similar to that of other serotype 1 human rotaviruses (greater than 98% homology).

In our previous study in which the cross-reactive neutralization epitopes on VP4 of human rotaviruses were analyzed, K8 was found to bear unique VP4 neutralization epitopes. This strain, which belongs to subgroup II and serotype 1, was not neutralized by any of six anti-VP4 neutralizing monoclonal antibodies which reacted with serotype 1, 3 and 4, or serotype 1 through 4 human rotavirus strains. Thus, the K8 strain appears to have a unique VP4 gene previously not described.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00451-04 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological and Molecular Characterization of Rotavirus Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visit. Assoc.	LID, NIAID
	Koki Taniguchi, Ph.D.	Visit. Assoc.	LID, NIAID
	Kazuo Nishikawa, M.D.	Visit. Assoc.	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visit. Scientist	LID, NIAID

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS 0	PROFESSIONAL 0	OTHER 0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1988-89

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00478-04 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Adenovirus Expression Vectors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visit. Scientist	LID, NIAID
Others:	Gisela Larralde	Guest Researcher	LID, NIAID

COOPERATING UNITS *(if any)*

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Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	.8	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Respiratory transmission of adenovirus types 4 and 7 is associated with the development of respiratory illness. However, enteric administration of these viruses produces a silent infection localized to the intestinal tract which is capable of preventing subsequent respiratory illness caused by these viruses. These observations served in earlier studies as the basis for the development in LID of the live enteric adenovirus type 4 and type 7 vaccines. An extensive experience in the use of these vaccines has been gained over the last 20 years since the vaccine is administered routinely to all new recruits in the military. The safety and efficacy of these adenovirus vaccines make them ideal candidates for use as vectors for insertion and expression of foreign genes.

We are testing this approach to develop recombinant adenovirus/rotavirus vaccines. Replication of such recombinants in the intestine may induce an immune response directed at the expressed rotavirus proteins and hence, it may confer resistance against rotavirus. The genes encoding the two outer surface proteins of rotavirus (gene 4 and gene 9) which are responsible for the induction of neutralizing antibody in the host, are being inserted into adenovirus vectors to construct such recombinants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00504-03 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Sequence of the Sixth Rotavirus Gene

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Robert M. Chanock, M.D.	Chief, LID	LID, NIAID

COOPERATING UNITS (*if any*)
BRB, NIAID (L. Maloy)

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Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

TERMINATED 1988-1989

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00507-03 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Molecular characterization of rotavirus serotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visit. Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

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Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Md 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Human rotavirus strains 69M and WI61 are distinct from human rotavirus serotypes 1, 2, 3, and 4 and from each other by plaque reduction neutralization and have been proposed as new human rotavirus serotypes (serotype 8 and serotype 9, respectively). The nucleotide sequence of the gene encoding the serotype-specific outer capsid glycoprotein, VP7, of strains 69M and WI61 was determined. In addition, the sequence of the VP7 gene of strain F45 (serotypically indistinguishable from WI61) was determined. Comparative analyses of the nucleotide and deduced amino acid sequences with those of reference strains from serotypes 1, 2, 3, 4, 5, and 6 demonstrated that WI61 and F45 share a high degree of sequence similarity with each other and that strains 69M, WI61, and F45 are distinct from established serotypes 1, 2, 3, 4, 5, and 6 in nine defined regions of the VP7 which are variable across rotavirus serotypes. Thus, sequence analysis confirms the existence of two additional human rotavirus serotypes.

In addition, sequence analysis is being utilized to determine whether vaccine efficacy is related to the circulating rotavirus strain. For example, rotavirus strains belonging to serotype 1 were obtained from Rochester, NY during a year when the RRV vaccine was protective and a year when it failed to protect against serotype 1 illness. This information will be important in determining whether antigenic variation due to genetic shift or drift is an important factor in the design of vaccine strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00510-02 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rotavirus VP7 Neutralization Epitopes of Serotype 3 Strains

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kazuo Nishikawa, M.D.	Visit. Assoc.	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visit. Assoc.	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visit. Sci.	LID, NIAID
	Koki Taniguchi, Ph.D.	Visit. Assoc.	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1988-1989

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00532-02 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Rotavirus Outer Capsid Protein VP4 by a Baculovirus Recombinant

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	Gisela Larralde	Guest Researcher	LID, NIAID
	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID
	R.M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.1	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A complete cDNA of the VP4 gene of porcine rotavirus strain OSU was inserted into a baculovirus expression vector under the control of its polyhedrin promoter. The VP4 outer capsid protein, which is a major neutralization antigen in rotavirus, was expressed in high yield in Spodoptera frugiperda (moth) cells. Reactivity of the expressed VP4 with polyclonal and monoclonal antibodies suggested that its neutralization epitopes were antigenically unaltered. Significantly, immunization of guinea pigs with VP4 expressed by the baculovirus recombinant induced antibodies, which inhibited hemagglutination of the OSU virus and neutralized its infectivity at high titer. These observations suggest that the VP4 expressed in moth cells maintained its native antigenic structure and thus, represents a source of authentic VP4 suitable for further biophysical and immunological studies. We have also expressed the VP4 of human KU rotavirus (serotype 1) in a baculovirus recombinant and preliminary evidence indicates that the expressed VP4 also retains its antigenic properties. Our success in expressing apparently authentic VP4 from two distinct rotaviruses suggests that it should be possible to express the VP4 of a wide variety of rotaviruses in authentic form and use these rotavirus outer capsid proteins to define: (1) the extent and distribution of VP4 polymorphism among strains recovered from humans and animals and (2) the role of VP4 in virulence and resistance to rotavirus infection and disease.

PHS 6040 (Rev 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00533-02 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Rotavirus Proteins with Monoclonal Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below tha Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20982

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
3.3	1.1	2.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced typa. Do not axceed the space provided.)*

A library of monoclonal antibodies specific for the two neutralization proteins of rotaviruses, VP7 (the major neutralization protein which determines serotype-specificity) and VP4 (the hemagglutinin) was developed in the LID. Efforts have continued to expand this library to include monoclonal antibodies which are needed for vital immunoassays and studies of the antigenic structure of the virus. Seven monoclonal antibodies specific for the new serotype 9 human rotavirus were isolated and characterized. These monoclonal antibodies exhibit high neutralizing activity against serotype 9 viruses but not against other rotavirus serotypes. One monoclonal antibody, 6A1, exhibits specific reactivity with serotype 9 viruses in ELISA and has been characterized extensively prior to its addition to the existing panel of monoclonal antibodies used for serotyping human rotavirus strains. A recently developed epitope blocking assay (which measures competitive inhibition of binding of Mabs to virus) now makes it possible to measure serotype-specific antibody responses of rotavirus vaccinees. For example, this technique has proven particularly helpful in measuring the antibody response of young infant vaccinees to major protective neutralization epitopes present on rotavirus vaccine strains. The most significant observation to emerge from these analyses was that young infants who lack serum antibodies to all rotaviruses respond to immunization with a serotype-specific response, whereas, infants with prior rotavirus experience develop a more broad heterotypic response. In addition, immunological correlates of resistance were identified when the VP4 and VP7 epitope blocking techniques were used to analyse sera from adult volunteers who were challenged with human rotavirus. Only volunteers who lacked serum antibodies to the VP7-specific epitopes of the challenge strain became ill when virus was fed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00534-02 LID

PERIOD COVERED
October 1, 1989 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Molecular Analysis of Nongroup A Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Yuan Qian, M.D	Visiting Associate	LID, NIAID
Others:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Although the major goal of the Epidemiology Section is the development of a vaccine against group A rotavirus, it is important to study other groups of rotavirus (B-E) in order to determine their importance as etiologic agents of human diarrheal disease. An immunoassay for the detection of human group B rotavirus which was previously not available in our laboratory, was developed in order to monitor the growth of virus in tissue culture and to facilitate epidemiologic studies. In addition, cloning strategies were developed which will allow significant progress in the molecular analysis of nongroup A rotaviruses. Thus far, efforts to grow group B rotavirus in tissue culture have not been successful.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00558-01 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prediction of Rotavirus Serotypes by Hybridization Specific to Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Gisela Larralde	Guest Researcher	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.6	0.5	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic probes capable of recognizing differences in the VP4 and VP7 genes of rotaviruses belonging to different serotypes have been derived from a variety of these genes and employed to identify the serotype specificity of rotavirus field strains. These probes were synthesized from either rotavirus RNA or cloned DNA copies of the fourth (VP4) or ninth gene (VP7) by amplification of specific regions (300-400 bases long) which were divergent among serotypes using the "polymerase chain reaction" (PCR). The correct serotype (VP4 and VP7) of several well characterized laboratory strains was confirmed using these probes.

The VP7 specificity of several rotavirus field strains from different sources was also identified with these probes. The results were similar to those obtained with monoclonal antibody ELISA. The VP4 specificities of the same field isolates have also been determined. This latter result is of importance since monoclonal antibodies are currently not available to classify the VP4 antigenic specificity of different rotavirus strains.

"Serotyping" rotaviruses by this approach may be of practical benefit in epidemiological studies and should increase our understanding the natural history of rotaviruses. It will also aid in the evaluation of rotavirus vaccine candidates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI00559-01 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Gene Segment 1 of the Gottfried Porcine Rotavirus Strain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Norio Fukuhara, Ph.D.	Visiting Fellow	LID, NIAID
Others:	Kazuo Nishikawa, M.D.	Visiting Assoc.	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Assoc.	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.1	1.0	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nucleotide sequence of gene segment 1 of the porcine rotavirus Gottfried strain (which shares neutralization specificity with human rotavirus serotype 4 via VP7) was determined. This gene segment encodes VP1 which is present in single-shelled rotavirus particles. Other scientists have suggested that VP1 provides virus transcriptase and replicase enzymatic activities. Gene segment 1 was shown to be 3302 nucleotides in length and had a single open reading frame capable of encoding a protein of 1088 amino acids. The Gottfried strain porcine rotavirus VP1 was observed to share 5 consensus sequences with RNA-dependent RNA polymerases of 8 different plant and animal single-stranded RNA viruses. Thus, our analysis suggests that VP1 plays a role in mRNA synthesis. In addition, VP3, which is also present in single shell rotavirus particles, exhibits GTP-binding activity. This suggests that VP3 has mRNA capping enzyme activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00560-01 LID

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The VP6 Gene of Equine Rotavirus Strain H-2 Encodes Two VP6 Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Mario Gorziglia, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: .3	PROFESSIONAL: .1	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Until now every rotavirus VP6 gene which has been characterized was shown to contain a unique AUG located 23 nucleotides downstream of its 5' terminus. An exception to this rule was recently observed. The VP6 gene of the equine H-2 rotavirus strain contains an additional AUG at residues 132-134, which initiates the translation of a second VP6 protein. Both VP6 products assume a trimeric conformation in the mature virion. The VP6 gene of the H2 strain is the second example of the presence of an active second AUG codon in rotavirus. The first example was previously described by others in the VP7 gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00561-01 LID

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Study of Genomic Rearrangement in Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	A.Z. Kapikian, M.D.	Head, Epidemiol. Sect.	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.1	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The complete nucleotide sequence of genomic double stranded RNA (dsRNA) segment 11, of the Alabama strain of rabbit rotavirus, was determined by sequencing dsRNA and mRNA using the dideoxy chain termination method. This strain has a "super short" RNA electrophoresis pattern. Its segment 11 is 1036 base pairs in length, 369 nucleotides longer than that reported for rotavirus strains with a "long" electropherotype. Sequence analysis indicated that the shift in genome segment 11 of Alabama strain to a "super short" RNA pattern (i.e., decreased mobility during electrophoresis) results from a simple duplication followed by a simple deletion.

Two potential open reading frames (orf) frames are present. The first orf, 594 base pairs in length (encoding 198 amino acids), extends from nucleotide 22 to a stop codon (TAA) at nucleotides 616 to 618. A stretch of 92 nucleotides (from positions 619-711) separate the end of the first and the beginning of the second orf. Sixty-five of these nucleotides (from bases 625 to 690) are similar in sequence to the nucleotides from bases 169 to 234. In addition, 18 nucleotides from positions 691 to 708 are similar in sequence to those at positions 322 to 339. The second orf of gene 11 of Alabama strain extends from nucleotides 712 to 984 and is 272 base pairs in length. Of interest, this orf is a duplication of the sequence corresponding to nucleotides 349 to 615 and is followed by a 3' noncoding region of 52 nucleotides. This second orf, unique to the gene 11 of the Alabama strain, is capable of coding for a protein of 91 amino acids.

LMM

LABORATORY OF MOLECULAR MICROBIOLOGY

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LABORATORY OF MOLECULAR MICROBIOLOGY

SUMMARY

The Laboratory of Molecular Microbiology (LMM) applies molecular and biological techniques to study the structure, function, and regulation of prokaryotic and eukaryotic genes. Programmatically, a wide range (murine retroviruses, the human immunodeficiency virus [HIV], yeast, and mycoplasmas) of microorganisms and their host cells are investigated. Members of the LMM are physically located in Building 4 on the Bethesda campus and in Building 550 at the Frederick Cancer Research Facility. Although the research activities conducted by the LMM staff are quite diverse, a common theme involves the characterization of genetic elements (both chromosomal and episomal) affecting the interaction of microorganisms and the cells they infect.

The past 6 years have witnessed a tremendous explosion of new information about the biology and molecular structure of HIV. Virtually all of this information has accrued from tissue culture infections of human peripheral blood lymphocytes or continuous T cell lines. We now know that, in addition to the *gag, pol,* and *env* genes present in other groups of replication competent retroviruses, HIV, like other members of the lentivirus subfamily, contains a number of other genes, many of which regulate the production of viral proteins during the replicative cycle. These include the *tat, rev, nef, vpu, vip,* and *vpv* genes. Studies focusing on the biology of HIV infection have demonstrated that T lymphocytes, expressing the CD4 molecule on their surface, can bind virus particles as the initial step in the replicative cycle. Other work has shown that infected tissue culture cells, synthesizing the viral envelope protein gp120, are able to induce the formation of syncytia and death of uninfected T4 lymphocytes.

HIV infection in man is characterized by months to years of inapparent disease before development of AIDS or AIDS-related syndromes. During this interval, free virus is extremely difficult to demonstrate. It is very likely that this inefficient expression of HIV reflects, in part, the presence of latent forms of virus in mononuclear cells. Populations of infected lymphoctes or macrophages may exist which harbor integrated, but inactive HIV proviruses. Such potential cell reservoirs of virus could initiate rounds of virion production if appropriately stimulated. Factors capable of activating dormant copies of proviral DNA might also contribute to disease progression. An animal model that simulates latent HIV infection of man could facilitate the study of relevant cofactors of disease progression. Toward this end, two types of transgenic mice carrying different portions of HIV proviral DNA have been constructed.

In one, four independent transgenic mouse lines were constructed containing the HIV LTR linked to the bacterial gene encoding chloramphenicol acetyl transferase (CAT). A common tissue pattern of CAT expression was observed in each of the four lines, with detectable activity present in the eye, heart, spleen, thymus, and tail. Low levels of CAT were present in circulating lymphocytes, but CAT activity in these cells could be augmented following treatment with the mitogen phytohemagglutinin. Likewise, the low levels of CAT expression detected in circulating monocytes were greatly increased when these cells were propagated in the presence of the cytokines CSF-1, Gm-CSF, IL-4, and IL-2. Furthermore, Langerhans cells recovered from skin showed higher levels of CAT activity than those observed in other cells of the monocyte/macrophage lineage, suggesting that LTR-directed expression may increase in proportion to the degree of differentiation.

In the second system, transgenic mice containing intact copies of HIV proviral DNA were constructed and maintained in a high-containment BL4 facility. Founder animals were found not to be viremic and remained healthy during a 10 month observation period. Following mating to a non-transgenic male, one of the founder animals (No. 13) gave rise to F_1 progeny that developed a disease syndrome characterized by marked epidermal hyperplasia, lymphadenopathy, splenomegaly, pulmonary lymphoid infiltrates, growth retardation, and death by day 25 of life. This syndrome was characterized by minimal expression of HIV as monitored by *in situ* hybridization and immunochemical analyses of fixed tissue specimens. Unequivocal expression could only be demonstrated in the affected skin. No detectable viral RNA or protein was present in lymph nodes, spleen, thymus, lung, brain, kidney, GI tract, or liver of diseased mice; however, infectious HIV, indistinguishable from parental virus by immunoblot analysis, was recovered from the spleen, lymph nodes, and skin of five of five affected animals. Immunologic studies revealed no depletion of the Leu3/T4 subset of mouse T cells in the diseased mice.

Two of the other six founder mice gave rise to F_1 progeny with high (11 to 20%) mortality rates. Since these animals did not develop a spontaneous disease, they were injected with mitomycin C or 5-azacytidine, agents previously shown to induce expression of endogenous retroviral sequences. One of the strains tested (No. 62) was extremely sensitive to both agents; virus could be recovered from nearly 90% of treated animals. As we attempted to extend these "induction" studies, our entire transgenic mouse colony, housed in the BL4 facility, was destroyed due to human error. We are currently in the process of constructing new transgenic mice harboring complete copies of the HIV provirus. In addition, the NIH Biosafety Committee has given us permission to conduct this type of experiment in a facility designed to contain mice which does not require the use of a Class 3 cabinet system. We intend to focus on agents which induce expression of virus/HIV proteins, since such a system would represent a model to assess potentially useful anti-viral agents.

Molecular biological and virological techniques have been employed to dissect structure/function relationships of different HIV genes and their role during virus replication. Our basic strategy has been to employ techniques of site-specific mutagenesis to alter individual HIV genes which are then introduced back into an isogenic replication-competent proviral DNA.

Envelope glycoproteins. An examination of the synthesis and processing of the HIV-1 envelope precursor glycoprotein gp160 revealed that only 5 to 15% is cleaved to produce the mature gp120 in infected human PBLs or in continuous T cell lines. Intracellular sorting results in the transfer of most uncleaved gp160 to lysosomes, where it is degraded, while gp120 is transported to the cell surface and subsequently secreted. Cleavage of gp160 to generate gp120 occurs intracellularly and can be inhibited by NH_4Cl. These results indicate that intracellular cleavage of gp160 determines the intracellular transport and survivial of the envelope glycoproteins necessary to produce infectious virus.

An amino acid substitution within the second conserved domain (residue 267) of gp120 results in the production of noninfectious virus. A detailed characterization of the mutagenized gp120 indicated no alteration in CD4 binding properties, but a reduced capacity to induce syncytia was consistently observed. Molecular characterization of "second site" spontaneous revertant viruses which arose during long-term co-cultures of the *env* mutant defined three discrete regions of gp120 (around residues 128, 267, and 308) which interact with one another during the early phases of productive viral infection. The critical step affected by these domains of the viral envelope occurs subsequent to the adsorption of virions to the cell surface and either prior to or concomintant with the fusion of viral and cellular membranes.

Molecular and biochemical analyses of the vpu protein. During the productive infection of human T cells by HIV, we have shown that *vpu* is post-translationally modified by phosphorylation, since a 16 kDa phosphoprotein can be immunoprecipitated with serum from an HIV positive individual and a *vpu*-specific antiserum. *In vitro* translation studies demonstrated that *vpu* is cotranslationally integrated into canine microsomal membranes, suggesting that it is an integral membrane protein. Although infected cells synthesize comparable amounts of gp160/120 and *vpu*, no *vpu* was detected in cell-free culture fluids, implying that it is *not* virion associated. Processing of viral precursor proteins was unaffected by the absence of *vpu*, and no differences were detected in the protein composition of wild-type and *vpu* mutant virions; however, the release of progeny virions from cultures producing *vpu*-defective virus was found to be delayed, resulting in the intracellular accumulation of viral proteins. These results suggest, therefore, that *vpu* mediates the release of progeny virus particles from infected cells. Studies are presently underway to interfere with *vpu* function and, thereby, impede HIV production.

Structure and function of the HIV long terminal repeat. The expression and replication of HIV is dependent on both viral and cellular factors. Many of the cellular and viral regulatory proteins exert their effects through *cis*-acting sequences situated in the long terminal repeat (LTR). The study of the function of these sequences in both acute and latent HIV infections provides an approach to analyze their function during the viral life cycle.

The role of LTR regulatory sequences in the activation of latent copies of the HIV provirus was examined in a cell line (ACH2) which harbors a single integrated copy of viral DNA which is expressed constitutively at very low levels. Treatment of ACH2 cells with the cytokine, tumor necrosis factor α (TNF-α), results in activation of HIV production. This induction is associated with increased steady-state levels of HIV RNA. Nuclear run-on analyses have shown that there is an increase in initiation of HIV transcription following exposure of ACH2 cells to TNF-α. Treatment of ACH2 cells with this agent was associated with the induction of nuclear factor(s) binding to the core enhancer/ NF-KB binding sites in the HIV LTR. No change was observed in levels of binding to the Sp1 or NFAT binding sites. Transfection assays employing HIV LTR-CAT plasmids showed that deletion of the NF-KB sites eliminated this TNF-α-induced activation.

The role of LTR regulatory sequences during acute replication of HIV in human T cells was also studied. These analyses were performed using a novel circularly-permuted form of our infectious molecular HIV clone which contained a single LTR. Mutations were introduced into this single LTR by site-specific mutagenesis procedures, and the altered LTR was reinserted into its isogenic parental proviral DNA. A provirus containing a deletion of both NF-KB binding sites was capable of replicating in a variety of human T cells/T cell lines. Replication was delayed in MT4 cells, but was indistinguishable from wild-type virus in PHA-activated normal human PBLs. An HIV derivative missing all three Sp1 binding sites was able to replicate in MT4 cells, but had no detectable biological activity in CEM cells. Virus missing the two NF-KB and the three Sp1 sites was replication incompetent. Similarly, a virus containing a 4 base-pair deletion in the TAR region which eliminates responsiveness to the HIV *tat* protein was also unable to replicate. Thus, a functional TAR region and at least some of the NF-KB and Sp1 sites are required for viral infectivity. The relative role of the NF-KB and Sp1 sites varies in different T cell lines. Viruses deleted in the Sp1 sites are able to replicate in cells such as MT4, which contain high levels of NF-KB activity; however, they are not infectious in cells such as CEM, which lack NF-KB activity.

Other Research Accomplishments

Biological and molecular features of pathogenic mycoplasmas. Mixed respiratory infections with *Mycoplasma pneumoniae* and *Mycoplasma genitalium* were first described from this laboratory; however, the well established serologic cross reactions that are observed between the two organisms (and other related features) suggest that *M. genitalium* strains in the respiratory tract are usually undetected in this relationship. We have now identified mixtures of the two mycoplasmas in an isolate from synovial fluid of a patient with acute polyarthritis secondary to respiratory infection. Each mycoplasma was distinguished by unique attachment proteins and on specific serologic tests on cloned and

purified isolates. Techniques are now available to successfully isolate and identify each of these two important pathogens and to establish what role *M. genitalium* may play in respiratory and/or joint infections.
(Tully, Baseman, Davis)

Evolution and classification of mollicutes. An ongoing international collaborative program devoted to new wall-less prokaryotes (class *Mollicutes*) has defined and characterized a number of new species from unusual environments. These new organisms have been included in a comprehensive phylogenetic analysis of over 40 mollicutes involving sequence similarities in evolutionarily-conserved 16S ribosomal RNA. Several of the new insect/plant-associated *Mycoplasma* species were shown to represent new descendant branches (clades) arising from more ancestral helical mollicutes (*Spiroplasma* species). New sterol-nonrequiring mollicutes from insect/plants were found to be phylogenetically distinct from the classic animal *Acholeplasma* species.
(Tully, Bove, Whitcomb, Woese)

Loss of HIV and MuLV viral infectivity by single amino acid changes in RT. Substitution of either of two conserved aspartic acid residues in the putative RNase H domain of HIV or Moloney RTs resulted in loss of ability to produce infectious virus. Defective viruses were produced as demonstrated by RT activity in culture supernatant fluids. The defect was identified in mutant RTs expressed in *E. coli* which had near normal polymerase activity, whereas RNase H activity was greatly diminished. These studies identify two functional amino acids in the RNase H domain and show that interference with RNase H activity effectively blocks the retroviral life cycle.
(Repaske, Hartley, Clavel)

Early DNA transcripts in host cells infected with RNase H defective M-MuLV. NIH 3T3 cells infected with RNase H defective virus were analyzed for proviral DNA transcription at various times after infection. With defective RNase H, only minus strand "strong-stop" DNA should be detected. At the earliest time point (15 min), both minus strand "strong-stop" and LTR DNA were found. Subsequent extension of minus strand DNA was arrested. These findings do not fit conventional concepts of the early events in the viral life cycle.
(Repaske, Silver)

A packaging mutant of HIV. A mutation was created in the HIV genome that removed 30 nucleotides in the region situated between the 5′ splice donor and the start of the *gag* open reading frame. The mutated provirus produces viral proteins and normal amounts of viral particles; however, the infectivity of progeny virus particles is greatly reduced, as is the amount of RNA inside the virions. We conclude, therefore, that this 30 base pair deletion affects the packaging of genomic RNA into HIV particles. Such a mutant should prove valuable in further studies on the mechanisms of RNA packaging of HIV particles, and will also be used in experiments involving HIV retroviral vectors.

(Clavel)

Genetic mapping of mouse chromosomal genes. Various genes have been mapped to mouse chromosomes by Southern blot analysis of DNAs from somatic cell hybrids containing different mouse chromosomes. Many of these genes were then positioned by Southern blot analysis of DNAs from an interspecific backcross. Newly mapped genes include those encoding the tumor rejection antigen gp96, a sequence rearranged with *Igk* in a melanoma, and the ecotropic MuLV receptor. These genetic systems have also been used to map other mouse genes, including a cDNA shown to be responsible for the *rds* mutation (retinal degeneration slow) and a brain-specific cDNA which maps to the site of a neurological mutation called *nr* (nervous).

(Kozak, Villar)

Characterization of endogenous viruses of the wild mouse *M. Spretus*. *M. spretus* (morocco) contains up to 10 MCF-related proviral genes. Although *spretus* does not contain detectable retrovirus-related RNA, inoculation of these mice with Moloney ecotropic MuLV results in the production of recombinant MCF virus. To examine transcriptional regulation of *spretus* proviruses and the role of transcription in recombination, we cloned one full-length *spretus* provirus and sequenced its LTR. This provirus contains a major deletion of 38 bases within U3 and numerous scattered base differences and small deletions that distinguish it from other U3 regions. This LTR was not able to promote CAT expression in transfection assays; however, deletion of all LTR sequences upstream of the TATAA box restored promotor activity, suggesting removal of a negative regulatory sequence. Further characterization of this LTR is in progress.

(Kozak, Villar)

Characterization of a novel wild mouse virus. HoMuLV is a mouse C-type virus with ecotropic host range isolated from *M. hortulanus*, a species indigenous to eastern Europe. It is not transmitted through the germline like its inbred mouse counterparts. The virus was cloned and partially sequenced and shown to differ from other viruses in its p12-*gag* and SU protein. The enhancer region of its LTR closely resembles that of Friend MuLV and includes many of the same protein binding sites. This similarity probably accounts for the ability of this HoMuLV, like Friend, to cause erytholeukemias.

(Kozak, Voytek)

PCR method for constructing recombinant DNA molecules without the need for homologous restriction sites. PCR allows the attachment of arbitrary short sequences (~ 20 nucleotides) to the ends of amplifiable DNA segments and the linking up of segments with homologous ends through annealing and primer extension reactions. Using these methods, we have constructed chimeric viral segments in which the regulatory regions from one virus (*e.g.*, the SV40 enhancer or the Moloney MuLV R region) are substituted for the corresponding portions of HIV. This method promises to have wide application in structure-function studies.

(Silver)

A novel recombinant murine retrovirus-like DNA element contains a 'solo' endonuclease gene. A novel endogenous murine retroviral-related DNA, designated B-13, was isolated from BALB/c mouse genome. Nucleotide sequence analysis indicated that B-13 DNA was distantly related to known infectious MuLVs and had a unique genomic structure: *1)* *gag* and *pol* sequences were present, but *env* sequences were absent; *2)* no 3′ LTR was seen, only a novel 5′ LTR was present; and *3)* the endonuclease gene, located in the 3′ *pol* region, was bounded by cellular sequences. Southern blot analysis of various mouse DNAs using B-13-specific DNA probes indicated the presence of several amplified copies of *gag* and 5′ *pol* sequences, but only one amplified endonuclease gene. Furthermore, the amplification of the 5′ sequences occurred prior to that of the endonuclease gene. These results suggest that B-13 DNA arose *via* recombination between a unique retrotransposon-like element with high amplification potention and a 'solo' endonuclease gene.

(Khan, Buckler)

Monoclonal antibodies against *ras* proteins. Monoclonal antibodies against intact *ras* proteins were prepared, and the region of the p21 molecule encompassing the epitope recognized by each antibody was determined. Two regions (amino acid stretches 23 to 69 and 89 to 106) accounted for the epitopes of 80% of the mAbs raised, indicating that those are the regions most exposed at the surface of *ras* and, thus, the most immunogenic. These mAbs exhibited significantly higher affinity than other known *ras* antibodies and made possible their use in applications such as the immunoaffinity purification of *ras* proteins and their immunohistochemical detection in fixed tissues.

(Nebreda, Alonso, Bryan, Santos)

ras gene products and altered pathways of cellular signal transduction. Malignant transformation mediated by cytoplasmic and membrane-associated oncogenes including *ras* results in pleiotropic alterations of phospholipid signaling pathways (*i.e.*, constitutive activation of PLA_2 and altered coupling [uncoupling or supercoupling] between external signals and internal PLC). These alterations are not due to altered function of receptors, G proteins, or effector enzymes but, rather, may involve faulty interactions between these otherwise normal components of the PI signaling pathway. These findings discount the notion that *ras* proteins are regulatory elements of PLA_2 and PLC, and suggest the existence of common biochemical pathways for transformation by cytoplasmic and membrane-associated (but not nuclear) oncogenes.

(Alonso, Santos)

ras proteins and meiotic maturation of *Xenopus* oocytes. Full grown *Xenopus* oocytes are arrested at the G2/M border of the first meiotic division. Progesterone, insulin, or microinjected *ras* proteins release this G2 block and lead to maturation involving germinal vesicle (nuclear membrane) breakdown (GVBD); however, inhibition of protein synthesis completely blocks progesterone and insulin-induced maturation but not *ras*-induced maturation. These results suggest that *ras* proteins act at a point close to activation of MPF, an activity common to all eukaryotic cells at the onset of division.

(Nebreda, Allende, Santos)

ras and *mos* gene products in meiotic maturation of *Xenopus* oocytes. In *Xenopus* oocytes, microinjection of *mos*-specific antisense oligonucleotides prevents expression of $pp39^{mos}$ and blocks meiotic maturation induced by microinjected *ras* oncogene proteins; however, *mos* antisense oligonucleotides did not block *ras*-induced maturation occurring in the presence of the protein synthesis inhibitor cycloheximide. These observations indicate that, under physiological conditions, *mos* function is required after *ras* in a common pathway inducing meiotic maturation, but the requirement is abolished in the absence of protein synthesis. The results are consistent with a model where the short-lived *mos* gene product acts as a negative regulator of another short-lived negative regulatory element of the pathway of meiotic maturation induced by *ras* proteins.

(Nebreda, Daar, Vandewoude, Santos)

Bacterial expression of HIV *nef*. A cDNA fragment coding for the *nef* gene of HIV was placed under the transcriptional control of the tightly regulated P_L promoter of bacteriophage lambda in the expression vector pRC23. Upon derepression of the PI promoter, large amounts (5 - 10% of total cellular protein) of the *nef* gene product are synthesized in *Escherichia coli*. We have purified the *nef* protein almost to homogeneity by a method involving solubilization with chaotropic agents followed by liquid chromatography. This purified *nef* preparation will be used to assess its biochemical properties.

(Ahmed, Venkatesan, Bryan, Nebreda, Santos)

Recombinant vaccinia viruses expressing the HIV-1 protease, p64 RT, p55, and p41 *gag* precursor proteins with or without protease have been constructed. Protein expression has been confirmed by immunological procedures. The protease expressed from vaccinia was also enzymatically active. Based on the protein sequence data, the DNA-encoding integration protein has been constructed and expressed in the vaccinia vector. A 15 kDa C-terminal cleavage product of p64 RT was found to possess RNase H activity, and the DNA corresponding to this protein has also been expressed in vaccinia. All these constructs are now being used to study the biochemical mechanisms of *gag-pol* processing.

(Venkatesan, Holland, Maitra)

Mechanism of action of the HIV-1 *tat* protein. The amino terminal 72 amino acids (coding exon 1) of the HIV-1 *tat* protein have been overexpressed in eukaryotic *Spodoptera frugiperda* cells using a baculovirus expression system. This 72 amino acid protein was shown to be biologically active when introduced into mammalian cells using a novel cell-cell fusion procedures. When presynthesized *tat* protein was introduced into mammalian cells, we found that activation of the HIV-1 LTR occurred in the absence of *de novo* cellular protein synthesis. This suggests that *trans*-activation by *tat* is mediated through pre-existing cellular transcriptional factors. We have identified three candidate cellular proteins (100, 62, and 46 kDa) that indeed bind to the HIV-1 TAR RNA with great avidity.

(Gatignol, Jeang)

The target for the HIV-1 *tat* protein is a nascent RNA molecule. Two types of mutations have been introduced into the HIV-1 TAR element. The first group consisted of point mutations within the +1 to +80 TAR nucleotides. The second group contained new sequences which were designed to interfere with formation of native TAR RNA secondary structure. Analyses of these mutants revealed that specific nucleotides between +22 to +24 and between +29 and +34 are important for *trans*-activation by *tat*. We also found that the formation, albeit transiently, of a native TAR RNA secondary structure is also required for *tat* responsiveness.

(Berkhout, Jeang)

HTLV-I *tax* protein activated the HTLV-I LTR through three consensus cAMP-responsive elements. Transcriptional activation of the HTLV-I LTR by *tax* requires a 21 bp sequence which is repeated three times within the HTLV-I viral LTR. We have verified that this 21 bp element is a cyclic-AMP (cAMP)-responsive target. We have found four cellular proteins in uninfected mammalian cells that bind to this consensus sequence. Depending on the context of flanking sequences, the *tax*-responsive 21 bp element can also be activated by phorbol ester. These findings suggest that the HTLV-I LTR is possibly regulated by the *tax* protein through the utilization of endogenous cellular signal transduction pathways.

(Semmes, Nelson, Jeang)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00027-22 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph G. Tully	Head, Mycoplasma Section	LMM, NIAID
Others:	David L. Rose	Research Microbiologist	LMM, NIAID

COOPERATING UNITS *(if any)* J.B. Baseman, Univ. of Texas, San Antonio; J.M. Bove, Univ. Bordeaux, France; R.F. Whitcomb, USDA, Beltsville, MD; C.P. Davis, Univ. of Texas, Galveston; C. Woese, Univ. of Illinois, Urbana.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Mycoplasma Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick MD 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	1	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New ecological information on *Mycoplasma genitalium*, developed in earlier collaborative studies, identified the organism in the human upper respiratory tract in close association with *M. pneumoniae*, an important respiratory disease pathogen of man. Continued characterization of the biological and molecular features of *M. genitalium* has led to improved techniques in detection and identification of the organism, particularly in throat specimens and other human clinical material (synovial fluid) where mixed infections have been found. Although data sufficient to establish *M. genitalium* as a respiratory or other human tissue pathogen are still lacking, these advances in identification of unique molecular components of both *M. genitalium* and *M. pneumoniae* provide methodology to unequivocally identify each mycoplasma and to assess host tissues for presence of species-specific antigens or nucleic acids and/or specific immune complexes. Efforts in understanding the evolution and classification of mollicutes were pursued in collaborative studies with a group at the Univ. of Illinois. A phylogenetic analysis on over 40 representative mollicutes, based upon sequence analysis of 16S rRNA, provided several new and novel evolutionary concepts. A number of new insect- or plant-derived *Mycoplasma* species recently characterized by our collaborative group were found to represent new descendent branches (clades) from helical mollicutes (spiroplasmas), while some new *Acholeplasma* species we identified from insects were found to be evolutionarily distinct from classic animal-derived *Acholeplasmas*. These findings also confirmed the rapid evolutionary changes occurring in mollicutes and the value of these organisms as models in assessing the possible role evolutionary rate plays in evolutionary change.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00190-11 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Martin	Chief	LMM, NIAID
Others:	J. Leonard	Medical Staff Fellow	LMM, NIAID
	B. Potts	Senior Staff Fellow	LMM, NIAID
	E. Vicenzi	Visiting Associate	LMM, NIAID

COOPERATING UNITS (if any) J. Abrumczuk and A. Notkins, LOM, NIDR; G. Shearer and F. Hakim, Immunology Branch, NCI; L. Lamperth, LEN, NINCDS

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6	3	3

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Transgenic mice were constructed to model human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) directed expression *in vivo*. One group of animals was constructed containing the HIV LTR linked to the bacterial gene encoding chloramphenicol acetyl transferase (CAT). Low levels of CAT were present in circulating lymphocytes, but CAT activity in these cells could be augmented following treatment with the mitogen phytohemagglutinin. Likewise, the low levels of CAT expression detected in circulating monocytes were greatly increased when these cells were propagated in the presence of the cytokines CSF-1, Gm-CSF, IL-4, and IL-2. Furthermore, Langerhans cells recovered from skin showed higher levels of CAT activity than those observed in other cells of the monocyte/macrophage lineage, suggesting that LTR-directed expression may increase in proportion to the degree of differentiation.

Another colony of transgenic mice was generated which carried complete, potentially infectious copies of HIV proviral DNA. Following mating to a non-transgenic male, one of the founder animals (No. 13) gave rise to F_1 progeny that developed a disease syndrome characterized by marked epidermal hyperplasia, lymphadenopathy, splenomegaly, pulmonary lymphoid infiltrates, growth retardation, and death by day 25 of life. This syndrome was characterized by minimal expression of HIV as monitored by *in situ* hybridization and immunochemical analyses of fixed tissue specimens. Unequivocal expression could only be demonstrated in the affected skin; no detectable viral RNA or protein was present in lymph nodes, spleen, thymus, lung, brain, kidney, GI tract, or liver of diseased mice; however, infectious HIV, indistinguishable from parental virus by immunoblot analysis, was recovered from the spleen, lymph nodes, and skin of five of five affected animals.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00218-08 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Biochemical and Chemical Studies on Retroviral RNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roy Repaske	Research Chemist	LMM, NIAID
Others:	Francois Clavel	Visiting Fellow	LMM, NIAID
	Sundararajan Venkatesan	Expert	LMM, NIAID
	M. David Hoggan	Senior Scientist	LMM, NIAID
	Ron L. Willey	Biologist	LMM, NIAID
	Malcolm A. Martin	Chief	LMM, NIAID
	Jonathan Silver	Medical Officer	LMM, NIAID

COOPERATING UNITS *(if any)* Janet Hartley, Research Microbiologist, LVD, NIAID; Richard J. Feldman, CR, DRT

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Six conserved amino acids were identified in an alignment of *E. coli* RNase H with the C-terminal region (putative RNase H domain) of reverse transcriptase (RT) of retroviruses. The functional significance of two conserved aspartic acid residues was determined by amino acid substitutions (modifying codons by site-directed mutagenesis) in Moloney murine leukemia virus (M-MuLV) and in human immunodeficiency virus (HIV). Transfected mutant proviruses produced noninfectious viruses in both systems when either aspartic acid was substituted. RTs expressed in *E. coli* containing these mutations were found to have near normal polymerase activity, but RNase H activity was severely depressed. Appropriate host cells infected with mutant viruses were analyzed for transcription of viral DNA. Absence of complete minus strand DNA was consistent with ineffective RNase H function. The degree of loss of RNase H function by single conserved amino acid substitutions suggests these amino acids have a functional role in RNase H.

A mutation was created in the HIV genome that removed 30 nucleotides in the region that lies between the 5′ splice donor and the start of the *gag* open reading frame. The mutated provirus produces normal viral proteins and normal amounts of viral particles; however, the infectivity of these particles is greatly reduced. The amount of RNA inside the mutant particles is also markedly reduced. We conclude that the mutation affects the packaging of the viral genomic RNA into HIV particles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00281-07 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Charles E. Buckler	Research Biologist	LMM, NIAID
Others:	Christine A. Kozak	Research Microbiologist	LMM, NIAID

COOPERATING UNITS *(if any)* None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	0

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Terminated.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00300-08 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Control of Resistance to Viral Leukemogenesis in Wild Mouse Populations

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Christine A. Kozak	Research Microbiologist	LMM, NIAID
Others:	Claudio Villar	Guest Worker	LMM, NIAID
	Peter Voytek	Visiting IPA Agreement	LMM, NIAID

COOPERATING UNITS (*if any*)

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Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Studies on the genetic basis of virus-induced neoplastic disease have led to the identification of many chromosomal genes involved in these processes. Although most of these studies rely on use of the various inbred strains, we have been examining wild mouse populations for infectious MuLVs and for susceptibility to virus-induced disease. We have identified a novel neoplastic MuLV, termed HoMuLV, found in the European wild mouse, *M. hortulanus*. HoMuLV, an ecotropic virus, was molecularly cloned and sequenced, and its SU and p12-*gag* proteins were shown to differ substantially from those of the known ecotropic MuLVs. Its LTR contains an enhancer element that most closely resembles that of Friend MuLV and, like Friend, HoMuLV is capable of inducing erythroleukemias. In another series of experiments, we have continued our studies on the germline proviruses of *M. spretus* (Morocco). *M. spretus* carries at least ten proviruses related to the MCF MuLV, but it fails to express detectable levels of retrovirus-related RNA; however, *M. spretus* mice will produce infectious recombinant MCF virus and develop lymphoma after inoculation with Moloney ecotropic MuLV. Thus, these mice contain the endogenous sequences which can contribute to the generation of leukemogenic MuLVs. In order to investigate the relationship between endogenous virus expression and recombination, one germline *spretus* provirus was molecularly cloned and its LTR sequenced and examined for promoter activity in CAT constructs. The sequence of this LTR differs from that of LTRs, and it shows no transcriptional activity; however, deletion of sequences upstream of the TATAA box restores promoter activity, suggesting the existence of a negative regulatory sequence in the upstream region.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00301-08 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Genes Involved in Transformation and MuLV Transmission in Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Christine A. Kozak	Research Microbiologist	LMM, NIAID
Others:	Beverly Mock	Senior Staff Fellow	NCI
	Ettore Appella	Chemist	NCI
	Frank Gonzalez	Acting Chief, Nucleic Acids Sec	LMC,NCI

COOPERATING UNITS *(if any)* S. Kistler, U. S. Car.; H. Orr, U. Minn.; P. Srivastava, Sloan Kettering; S. Chen, Mass. General; H. Zachau, Inst. for Physiologische Chemie der Universitat, Munich; D. Farber, UCLA; C. Lau, UCSF; G. Travis, Scripps Clinic; I. Young, Australian Natl Univ.; J. Stone, Jackson Laboratory.

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1	1	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many genes have been identified in the laboratory mouse which affect retrovirus-induced disease. We have identified and mapped a number of these genes to specific mouse chromosomes by the analysis of Chinese hamster X mouse somatic cell hybrids, each of which contains different mouse chromosomes. In many cases, these genes have also been localized to specific chromosomal positions by the analysis of the progeny of an interspecific backcross. These studies have resulted in the chromosomal mapping of the genes encoding the ecotropic MuLV cell surface receptor, the tumor rejection antigen gp96, a sequence involved in an *Igk* rearrangement in MOPC cells, and genes for the lymphokines Il-5 and Il-6. In addition to these studies, we have also used this approach to map genes related to heat shock proteins, actin genes, and genes which may be responsible for a variety of inherited disorders of the mouse such as retinal degeneration, markers for Huntington's disease, the mouse homologue of the human retinoblastoma gene, and sex-determining factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00304-08 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Retroviral Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jonathan Silver	Medical Officer	LMM, NIAID
Others:	Vijaya Keerikatte	Technician	LMM, NIAID
	Franz Sels	Guest Researcher	LMM, NIAID
	Jim Felser	Medical Staff Fellow	LMM, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Microbiology

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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	3	0.25

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  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We continued our studies of proviral integration using "inside-out" PCR (polymerase chain reaction) to amplify cellular DNA flanking integrated proviruses in hematopoietic malignancies induced by Friend and Moloney murine leukemia viruses (MuLVs). The results to date are inconclusive, in that we have not verified whether the tumor-specific amplification products represent legitimate flanking sequences. Experiments to resolve these questions are in progress. We applied PCR to analyzing early events in retroviral replication following infection of mouse cells with wild-type Moloney MuLV and RNase H mutants of Moloney MuLV. Preliminary results suggest that the RNase H mutants are unexpectedly deficient in reverse transcriptase (RT) activity as well, in that there appears to be a decreasing gradient of RT products from moderately reduced amounts of U3 DNA, dramatically reduced amounts of *gag* DNA, and undetectable amounts of double LTR circles. We developed a new method of constructing recombinant DNA molecules using PCR which does not require the presence of homologous restriction sites. Using this method, we substituted segments of Moloney MuLV (R region of the LTR) and SV40 (enhancer region) for corresponding regions of HIV to investigate the functions of these regulatory regions of HIV. This recombinant DNA method using PCR should have wide applicability, and we are using it to study determinants of other viral phenotypes including retroviral host range.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00353-07 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural and Functional Studies of Mammalian Endogenous Retroviral Sequences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arifa S. Khan	Research Microbiologist	LMM, NIAID
Others:	Charles E. Buckler	Research Biologist	LMM, NIAID
	Richard G. Cuttler	Research Chemist	IRP, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

All mammalian genomes contain multiple copies of retroviral-related sequences. A novel endogenous murine retroviral-like DNA, designated B-13, was isolated from BALB/c mouse genome. Nucleotide sequence analysis indicated that B-13 DNA was distantly related to known infectious MuLVs and had a unique genomic structure: *1) gag* and *pol* sequences were present, but *env* sequences were absent; *2)* no 3′ LTR was seen, only a novel 5′ LTR was present; and *3)* the endonuclease gene, located in the 3′ *pol* region, was bound by cellular sequences. Southern blot analysis of various mouse DNAs using B-13-specific DNA probes indicated several amplified copies of *gag* and 5′ *pol* sequences, but only one amplified endonuclease gene. Furthermore, the amplification of the 5′ sequences occurred prior to that of the endonuclease gene. These results suggest that B-13 DNA arose *via* recombination between a unique retrotransposon-like element with high amplification potential and a 'solo' endonuclease gene.

Expression of endogenous retroviral and cellular genes was studied in brain, liver, and kidney tissues of inbred C57BL/6 mouse, *Mus musculus* wild mouse, and man, ranging in age from embryo to old. Retroviral sequences were expressed throughout life in a tissue-dependent manner. In case of both mouse and man, a similar kinetic of expression was seen with aging. The kinetics of expression of cellular genes was distinct from retroviral gene expression. Each gene (GAPDH, histone H2B, and β-actin) was expressed in a distinct age- and tissue-dependent manner. Furthermore, the kinetics of expression of the H2B gene was different in mouse and man.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00415-05 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. A. Martin	Chief	LMM, NIAID
Others:	K. Strebel	Visiting Associate	LMM, NIAID
	T. Klimkait	Visiting Associate	LMM, NIAID
	R. Willey	Biologist	LMM, NIAID

COOPERATING UNITS *(if any)* R. Klausner and J. Bonifacino, CBMB, NICHHD

LAB/BRANCH
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SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5	3	2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

An examination of the synthesis and processing of HIV-1 envelope precursor glycoprotein gp160 revealed that only 5 to 15% is cleaved to produce the mature gp120 in infected human PBLs or in continuous T cell lines. Intracellular sorting results in the transfer of most uncleaved gp160 to lysosomes, where it is degraded, while gp120 is transported to the cell surface and subsequently secreted. These results indicate that intracellular cleavage of gp160 determines the intracellular transport and survivial of the envelope glycoproteins necessary to produce infectious virus.

An amino acid substitution within the second conserved domain (residue 267) of gp120 results in the production of noninfectious virus. Molecular characterization of "second site" spontaneous revertant viruses which arose during long-term co-cultures of the *env* mutant defined three discrete regions of gp120 (around residues 128, 267, and 308) which interact with one another during the early phases of productive viral infection. The critical step afected by these domains of the viral envelope occurs subsequent to the adsorption of virions to the cell surface and either prior to or concomintant with the fusion of viral and cellular membranes.

Although infected cells synthesize comparable amounts of gp160/120 and *vpu*, no *vpu* was detected in cell-free culture fluids implying that it is *not* virion associated. The release of progeny virions from cultures producing *vpu*-defective virus was found to be delayed, resulting in the intracellular accumulation of viral proteins. These results suggest, therefore, that *vpu* mediates the release of progeny virus particles from infected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00437-05 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Biology and Genetics of the AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. D. Hoggan	Senior Scientist	LMM, NIAID
Others:	G. Englund	Biologist	LMM, NIAID
	M. A. Martin	Lab Chief	LMM, NIAID
	B. Potts	Senior Staff Fellow	LMM, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing studies of the morphological and biological characteristics of different HIV strains in various T and B cell lines, as well as different primary cell preparations, have been carried out. It has been shown that three different infectious molecular clones of AL-1 have distinctive biological properties in terms of growth kinetics, level of virus production, cell killing, and plaque morphology. Genotypic differences in the LTRs of the three clones accounted for the major differences in the biological properties of the AL-1 clones.

EM studies of HIV-infected bone marrow cultures demonstrated that HIV replication occurred primarily in promonocytes, with virus seen budding into cytoplasmic vacules. On occasion, multinucleated syncytia were seen containing large aggregates of HIV particles.

Attempts to produce specific polyclonal neutralizing antibody in guinea pigs and rabbits were not successful; however, it is of interest that sera from hyperimmune rabbits did stimulate virus production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00438-05 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Biology of Cellular Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Santos	Visiting Scientist	LMM, NIAID
Others:	T. Bryan	Microbiologist	LMM, NIAID
	T. Alonso	Guest Researcher	LMM, NIAID
	A. R. Nebreda	Visiting Fellow	LMM, NIAID

COOPERATING UNITS *(if any)* Ira Daar, NCI, FCRF; George Vandewoude, NCI, FCRF

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It has been suggested that *ras* proteins participate in transduction of cellular proliferative signals; however, their specific role is still undefined, and we and others have shown that they are not regulatory elements of any of the two main signaling pathways in mammalian cells, *i.e.*, the adenylate cyclase- and the phosphoinositide pathways. Recently, we have also shown that malignant transformation mediated by cytoplasmic and membrane-associated oncogenes including *ras* involves common biochemical pathways that result in pleiotropic alterations of phospholipid signaling pathways (*i.e.*, constitutive activation of PLA_2 and altered coupling [uncoupling or supercoupling] between external signals and intracellular PLC). These changes are not due to altered function of receptors, G proteins, or effector enzymes but, rather, involve faulty interactions between otherwise normal components of the different signaling pathways. Microinjected *ras* oncogene proteins can induce meiotic maturation of *Xenopus* oocytes. We have recently found that microinjection of *mos*-specific antisense oligonucleotides prevents expression of pp39 *mos* and blocks meiotic maturation induced by microinjected *ras* proteins; however, in the absence of protein synthesis, *ras* proteins still induce meiotic maturation, and *mos* antisense oligonucleotides do not block *ras*-induced maturation. In addition, *ras*-induced maturation in the presence of cycloheximide is specifically associated with phosphorylation of specific cellular substrates.

Our results in mammalian and amphibian cells suggest the occurrence of a common biochemical pathway for transduction of mitogenic and proliferative signals where the biochemical activities of membrane-associated proto-oncogene products (*src*-like or receptor-like tyrosine kinases) precedes those of the *ras* gene products and these, in turn, are followed by the participation of cytoplasmic serine or threonine kinases such as *mos* or *raf*. These studies suggest also that this common pathway involves a cascade of phosphorylation events where *ras* proteins exert, directly or indirectly, a key regulatory role.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00467-04 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Polypeptides Encoded by AIDS RV Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Venkatesan	Expert	LMM, NIAID
Others:	N. Ahmad	Visiting Fellow	LMM, NIAID
	R. J. Mervis	Microbiologist	LMM, NIAID
	E. Lillehoj	Scientist E	FCRC, NIAID

COOPERATING UNITS *(if any)* M.G. Raum, BRB, NIAID; H. Chan, Syntex Corp.; F.H. Rick Salazar, Syntex Corp.; T. Fuerst, MVI, Gaithersburg, MD; R. Willey, LMM, NIAID

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have purified and sequenced *pol* ORF-encoded proteins from HIV-1-infected cells and purified virus. The p64 and p51 RT (reverse transcriptase) and p34, the putative integrase that maps near the C-terminus of the *pol* ORF, were sequenced in this manner. We have also sequenced a p10 *gag-pol* protease responsible for the processing of the *gag* and *gag-pol* fusion protein. A sensitive assay for the protease using defined oligopeptides has also been developed.

Seven human immunodeficiency virus (HIV) *gag* polypeptides were identified in the extracellular virus and in infected CD4$^+$ lymphocytes by peptide mapping and limited amino acid sequencing. Two *gag* precursor proteins of 55,000 (p55) and 41,000 (p41) daltons were rapidly labeled and readily processed into the major internal *gag* proteins that were aligned within the *gag* ORF as NH2-p16(MA)-p24(CA)-p9(NC)-p7-COOH. Myristoylated p16(MA) protein was processed from the myristoylated p55 *gag* precursor protein. Both the mature p24(CA) and p16(MA) proteins were phosphorylated at *Ser* residue(s). Two forms of *gag* p41 species, one from the C-terminal processing of p55 and the other from N-terminal processing of p55 or from *de novo* synthesis initiating at an internal *Met* at position 142 of the *gag* ORF were identified

Recombinant vaccinia viruses expressing the HIV-1 protease, p64 RT, p55, and p41 *gag* precursor proteins with or without protease have been constructed. Protein expression has been confirmed by immunological procedures. Based on the protein sequence data, the DNA encoding integration protein has been constructed and expressed in the vaccinia vector. A 15 kDa C-terminal cleavage product of p64 RT was found to possess RNase H activity, and the DNA corresponding to this protein has also been expressed in vaccinia. All these constructs are now being used to study the biochemical mechanisms of *gag-pol* processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00526-01 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
To Investigate the Effects of the Expression of HIV Envelope Gene in CD4$^+$ Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Silver	Medical Officer	LMM, NIAID
Others:	J. Felser	Medical Staff Fellow	LMM, NIAID
	T. Klimkait	Guest Researcher	LMM, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Microbiology

SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00527-02 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning and Characterization of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. S. Theodore	Research Microbiologist	LMM, NIAID
Others:	M. A. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Heterogeneity among AIDS retroviral genomes has been clearly demonstrated. We have used molecular cloning, restriction enzyme analysis, and nucleotide sequencing techniques to characterize isolates of the human immunodeficiency virus (HIV). These studies may provide data regarding their structural as well as geographic diversity and, possibly, the degree of antigenic variation and pathogenicity among different HIV isolates. An African isolate (Zaire-2) and a North American isolate (NY-5) of HIV-1 were molecularly cloned using lambda bacteriophage vectors. Each clone contained a single LTR (9.2 kb genome) and, upon transfection of lambda clones into lymphocytes, infectious virus particles were produced. Reverse transcriptase (RT) activity resembled that of the constructed infectious clone (pNL4-3) and, upon passage of supernatants onto fresh lymphocytes, displayed similar RT kinetics of pNL4-3. The complete nucleotide sequence of each of these infectious molecular clones has now been analyzed, and attempts to prepare antisera of each isolate for neutralization tests are in progress.

A T cell clone (ACH-2) from T cells infected with HIV-1 produced HIV-1 in response to stimulation with phorbol myristic acetate (PMA). This isolate was molecularly cloned and, upon transfection into lymphocytes, produced virus; however, only in the presence of PMA. Passage onto fresh lymphocytes produced virus as monitored by RT in the absence of the inducing PMA. Presently, we are studying the biological role of PMA in viral induction of HIV-1 and the relationship of latency in viral infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00528-02 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Interactions of HIV Regulatory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sundararajan Venkatesan	Expert	LMM, NIAID
Others:	Nafees Ahmad	Visiting Associate	LMM, NIAID
	Ratan K. Maitra	Visiting Fellow	LMM, NIAID
	Steven M. Holland	NRC Res. Assoc.	LMM, NIAID

COOPERATING UNITS *(if any)* Clifford M. Lane, Medical Officer, LIR, NIAID; Peter Dickie, Staff Fellow, NIDR; Thomas M. Fuerst, Staff Scientist, MVI, Gaithersburg, MD; Scott Koenig, Medical Staff Fellow, LIR, NIAID

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The replication of human immunodeficiency virus, type 1 (HIV-1) requires the concerted action of two viral *trans*-activators, *Tat* and *Rev*, and is, in turn, moderated by the virus-coded transcriptional repressor, *Nef*. Rev-Nef- double mutant was also replication negative but had normal *Tat* function. Complementation of Rev- with *Rev* caused a decrease of synthesis of *Nef* accompanied by enhanced synthesis of viral structural proteins. The ability of *Rev* to modulate *Nef* solely from the provirus and relieve the inhibition of LTR transcription reveals a delicate balance of the functions of these two proteins.

Rev is essential for the expression of mRNAs containing a *Rev* responsive element (RRE) of about 236 nt in the HIV *env* ORF. To obtain large quantitites of *Rev*, a TK^- vaccinia virus recombinant containing the Rev gene under the control of T7 promoter inserted in the vaccinia TK locus was constructed. In coinfection experiments with vaccinia virus encoding T7 RNA polymerase, 2 - 3 mg of Rev was obtained from 10^9 cells, and the protein localized to the nucleus shortly after synthesis.

With increasing amounts of *Nef*, there was a dose-dependent decrease of transcription from the HIV LTR. *Nef*-expressing T lymphoid cell lines repressed LTR activation. *Nef*-expressing vaccinia virus, vTFNnef, was used to infect EBV immortalized AIDS patient T4 lymphocytes which were then used as targets for CTLs. Earliest CTL response was identified against *Nef*, and two important epitopes were mapped by peptide competition experiments. HIV-1 *nef* gene was associated with fetal death in transgenic mice, predominantly at the 6- to 8-day stage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00546-01 LMM

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Human Retrovirus Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. B. Rabson	Expert	LMM, NIAID
Others:	W. J. Maury	IRTA Fellow	LMM, NIAID
	C. Parrott	Guest Researcher	LMM, NIAID
	E. Duh	Guest Researcher	LMM, NIAID

COOPERATING UNITS *(if any)* M.A. Martin, LMM, NIAID; J. Leonard, E. Vicenzi; E. Ross, LMM, NIAID; T. Folks, Retrovirus Diseases, Centers for Disease Control; A. S. Fauci, LIR, NIAID; A. Buckler-White, Georgetown University

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is the understanding of the role of viral-cellular interactions in the regulation of gene expression of the human immunodeficiency virus (HIV). In particular, we have examined the role of *cis*-acting DNA sequences in the HIV long terminal repeat (LTR) in the control of HIV gene expression in both chronic or latent infections and in acute infection of human T lymphocytes. In the ACH2 cell line, a model of chronic HIV infection, HIV expression can be activated by treatment with the cytokine, tumor necrosis factor α (TNF-α). TNF-α activates HIV through induction of the cellular transcriptional factor, NF-KB, that binds to the HIV LTR. We have also studied the effect of the LTR regulatory sequences on the acute viral replication by mutating regulatory sequences in the LTR of an infectious molecular clone. These studies have shown that the NF-KB binding sites are not essential for HIV replication in human T cells. The Sp1 binding sites are not required for HIV replication in some T cell lines; however, they seem to be required for viral growth in T cells lacking the NF-KB protein. These studies have also shown that a mutation in the TAR region that blocks *tat* transactivation destroys HIV infectivity. Thus, the different protein binding sites in the LTR may play distinct roles in the infection of different human cells; however, the interaction of *tat* and TAR is critical for HIV infectivity. Finally, recent studies in our laboratory have identified the presence of a DNA sequence in the HIV *tat* coding region that functions as an enhancer element in a variety of human cells. The importance of this sequence in HIV replication has not been determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00547-01 LMM

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanism(s) of the HIV-1 *tat* and the HTLV-I *tax* Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.-T. Jeang	Senior Staff Fellow	LMM, NIAID
Others:	B. Berkhout	Visiting Associate	LMM, NIAID
	A. Gatignol	Visiting Fellow	LMM, NIAID
	O. J. Semmes, IV	Adjunct Scientist	LMM, NIAID
	N. Nelson	Chemist	LMM, NIAID
	M. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have overexpressed the HIV-1 *tat* and the HTLV-I *tax* proteins using an eucaryotic baculovirus-insect cell system. In analyzing the molecular mechanism(s) of *tat* and *tax*, we have noted the following salient observations: *1)* neither *tat* or *tax* appears to function through direct binding to its respective target sequence in the viral LTR; *2)* activation of the respective LTRs by the HIV-1 *tat* or the HTLV-I *tax* proteins occurs in the absence of *de novo* cellular protein synthesis; *3) tax* activates the HTLV-I LTR through a set of triply-repeated 21 bp sequences which contain a consensus cAMP-responsive motif; and *4)* a number of host cell proteins specifically bind to the HIV-1 TAR RNA sequence element.

Our current working hypothesis is that activation of the HIV-1/HTLV-I LTR is mediated through functional interactions between preexisting cellular transcription factors and the viral *trans*-activator proteins.

PHS 6040 (Rev 1/84) GPO 914-918

LPD

LABORATORY OF PARASITIC DISEASES
1989 Annual Report
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Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Disease
Summary - October 1, 1988 - September 30, 1989

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

The move of LPD from Building 5 to Building 4 was completed over a period of several months. In spite of the hard work and great efforts on the part of many who organized and carried out the move, it must be candidly stated that the engineering and construction of the newly renovated building left much to be desired. There have been many engineering defects that keep coming to attention (such as air handling), and shoddy construction is readily evident. LPD Staff has been remarkably patient during all of this and Intramural Administration have done their best to be helpful. Some late changes in personnel plans as to who would move and where resulted in inconvenience for some of the Staff; e.g. Drs. Cheever, Weinbach and Diamond are in the B2 basement level and Dr. Mercado is at the Twinbrook facility.

The largest void created by departing professional personnel this past year was that of Michael Good who returned to Australia for a Staff position at the Queensland Medical Research Institute -- we miss him greatly. Vidal de la Cruz found an offer from a local new biotechnology firm too attractive to refuse. Dr. Lee returned to Sweden for a research position and C. Syin took a position with the FDA. Lindsey Panton has a research job at Harvard and Altaf Lal has position with the malaria research group at CDC. Ann de Groot goes into an Infectious Disease Fellowship at Tufts. P. Sinnis, a Howard Hughes Fellow, returned to Medical School, as did F. Dontfraid. Ken Vernick finished his Ph.D. at Maryland as well as his part-time job with us, moving on to a post-doc at Carnegie Institution. Joe Vinetz, another Howard Hughes Fellow returned to medical school. Among the clinical types, Med. Staff Fellow David Freedman left to take a position at the University of Alabama. Peter Melby, a NRSA Fellow starts a clinical I.D. Fellowship a the University of Texas at San Antonio. The husband and wife team of Juan Carlos Engel and Patricia Doyle returned to research positions in Argentina. Anita Aggarwal continues her research on intestinal protozoa at Uniformed Services. Renu Lal, Altaf's wife, also has a job with her husband at CDC. Katie Pastakia finished her Sn. Staff Fellowship. Rosangela da Silva returned to a research job in Brazil. Steve Fuhrman finished his LCI Medical Staff Fellowship with Keith Joiner and goes into an Emergency Medicine training program. Ken Colina was another Howard Hughes Fellow who returned to Medical School.

Professional replacement in the Malaria Section include Steve Dolan, a Medical Staff Fellow from LCI, and Annie-John Walker, a recent Ph.D., and Cecelia Graziosi, a Special Volunteer, to work with Tom Wellems. Dr. X. Fang, a Chinese Visiting Fellow comes to Lou Miller's lab; Glen McConkey, a recent Ph.D., joins Tom McCutchan; a new NRSA Fellow, David Rawlings, started work with David Kaslow; and Marcel Huber is a new Visiting Fellow from Switzerland working with the entomology group. Dr. Peter Perlman, a well-known Swedish immunologist, is associated with the Malaria Section as a Fogarty Scholar, and his wife, Hedvig, also an immunologist, is working in the Section. Two new people joined Dr. Nash's lab, Bruno Gottstein, a Guest Worker from Switzerland, and Mike Mowatt, a Sn. Staff Fellow from Rockefeller University. Another Visiting Fellow from China, Dr. Y. Xu, a pathologist, joined Dr. Cheever. Dr. Sher's lab received a Guest Researcher from Dr. Capron's lab in France, Jean-Marie Grzych. Two new people joining Eric Ottesen's lab included Edgar Lobos, a Visiting Fellow, and Ajit Limaye, a Howard Hughes Fellow. Dr. T. Nozaki is a new Visiting Fellow from Japan with Dr. Dvorak. The husband and wife Bhattacharya team of Alok and Sudha came again from India for a few months to work with Dr. Diamond. Two post-docs from Brazil, Elvira Saravia and Gloria de Lima came to work a few months with David Sacks.

Dr. Keith Joiner and most of his group who make up the Unit on Microbial

Pathogenesis will move to Yale Medical School in the fall of 1989. Dr. Joiner becomes the Head of Infectious Diseases at Yale. Dr. Lee Hall will spend another year as in LCI Medical Staff Fellow on TDY at Yale.

In addition to a good deal of foreign travel for scientific meetings and on behalf of international organizations such as W.H.O., LPD staff were also active in the field in research activities. Dr. Neva and Al Gam spent several weeks in Zaire to investigate the role of strongyloidiasis as an opportunistic infection in AIDS patients. Drs. Nutman, Freedman, Klion and Cathy Steel made several trips to Guatemala on their onchocerciasis project. Dr. Ottesen traveled to India and Brazil as an advisor on Ivermectin treatment of lymphatic filariasis. Dr. Melby did some immunologic studies on leishmaniasis patients in collaboration with Dr. Ponce in Honduras and Dr. Neva followed up on this later in the same country. Dr. Diamond was in both India and Israel for his advisory and collaborative activities with workers in those countries. Ann DeGroot spent 1 1/2 months at the British Research Council Lab in the Gambia, West Africa, working on malaria.

HONORS AND AWARDS

All of the permanent professional staff, and many of the more senior non-tenured staff are involved in reviewing manuscripts for research journals. Also, many of them are on the Editorial Boards of one or more journals. This certainly should be regraded as recognition of their scientific standing by professional peers. Also many of the Staff serve on Ad Hoc Committees for granting agencies or foundations, and are invited participants to workshops and scientific meetings. For the most part these will not be individually cited, except for certain instances which are felt to deserve special recognition as mentioned below:

Dr. Louis Miller served as the President (1989) of the American Society of Tropical Medicine and Hygiene.

Dr. Theodore Nash was the recipient of the Outstanding Service Medal of the USPHS.

Dr. David Sacks received the NIAID Director's Merit Award in the fall of 1988, and was the recent recipient of the Chalmer's Medal of the Royal Society of Tropical Medicine and Hygiene of England. David also serves on the NIAID Tropical Medicine and Parasitology Study Section.

Dr. F. Alan Sher was selected to serve on the Burroughs-Wellcome Molecular Parasitology Fellowship Committee.

Dr. Eric Ottesen continues to serve on the Medical Advisory Board of the Edna McConnell Clark Foundation.

Dr. Dennis Dwyer set something of a record for being on Editorial Boards of three different journals, and serving on review panels of 4 or 5 different organizations, including W.H.O., a Basic Research Foundation of Israel Academy of Science, and the U.S. National Science Foundation!

Research Progress

CELL-MEDIATED IMMUNITY IN LEISHMANIASIS

More on T-cell subsets involved in leishmanial infections of mice. Last year's report described the finding that different antigenic fractions of L. major stimulated either TH1 or TH2 subsets of T-cells. Further, a T-cell clone was established that could passively confer protection to challenge infection. Now, additional T-cell lines have been established which give a consistent pattern of results; TH2-type cells are non-protective and produce mainly IL-4 and IL-5. By contrast, T-cell lines produced from animals immunized with the protective fraction are TH1 and they produce mainly IL-2 and INF-gamma. One of the protective T-cell clones recognizes a 10,000 M.W. protein from L. major by T-cell immunoblotting. Furthermore, the clone recognized antigens from several other species of Leishmania, but not antigens from trypanosomes (Scott, Pearce and Sher with Coffman from DNAX).

How do TH2 cells interfere with leishmanial immunity? Supernates derived from TH2 cell lines had the capacity to downregulate the INF-gamma mediated macrophage activation necessary for parasite killing. The effects of the TH2 supernates can also be inhibited by specific anti-lymphokine monoclonal antibodies. Thus, macrophages that normally would be expected to be activated to kill intracellular leishmania may be downregulated by IL-3, IL-4 and GM-CSF (Scott, Pearce, Sher and Coffman of DNAX).

Do T-cells recognize antigen directly on infected human macrophages? Since the intermediary role of other cell types, such as B cells or uninfected macrophages, in presenting antigen to T-cells cannot be excluded, a system was developed to try to answer this question. The technique involves infection of peripheral blood mononuclear cells from patients with leishmanial infection, then "light" fixation to stop further parasite antigen processing or release, and then addition of autologous T-cells to observe proliferative responses. With this system it appears that Leishmania infected macrophages are able to process and present antigens to sensitized T-cells (Sacks, Melby and De Lima).

No obvious cell-mediated abnormalities found in patients with atypical cutaneous leishmaniasis due to visceral organism. Some of the patients with the atypical non-ulcerative cutaneous leishmaniasis in Honduras caused by L.d. chagasi were evaluated for cell-mediated immune responses that might help explain their unusual and limited clinical expression of disease. In general, their responses were similar to patients with regular cutaneous leishmaniasis. More cases of this mild and localized cutaneous disease due to an organism that usually causes kala-azar, a systemic disease, continue to occur and the pathogenesis remains obscure (Melby, Neva, and Ponce in Honduras).

ROLE OF CYTOKINES IN SCHISTOSOME IMMUNITY

Role of IL-5 in schistosome induced eosinophilia and granuloma formation. For many years the classical model for study of immunological mechanisms responsible for eosinophilia and granuloma formation around eggs has been the schistosome infected mouse. It is now known that one of the products of T-cell stimulation, IL-5, is a cytokine that mediates the production of eosinophils. With the availability of monoclonal antibodies (Mabs) that can abrogate the action of cytokines it was possible to treat schistosome infected mice to assess the consequences of preventing blood and bone marrow eosinophilia. Granulomas around S. mansoni eggs in infected mice treated with anti-IL-5 Mabs were totally lacking in eosinophils, but were normal in size and went on to show fibrosis. In contrast, infected mice treated with Mabs vs. IFN-gamma had normal blood and tissue eosinophil levels as well as normal granulomas (Cheever, Scott, Sher and Coffman of DNAX).

Effect of cytokine depletion on protective immunity. To test the role of specific cytokines in immunity, mice vaccinated with irradiated S. mansoni cercariae were treated with Mabs vs. either IL-5 or INF-gamma. Although IL-5 depleted animals had no eosinophils, protective immunity was not affected. In contrast, the immunized and infected mice treated with anti-IFN-gamma showed a partial though reproducible reduction in immunity. These findings support the concept that protective immunity against schistosomiasis in mice is a function of TH1 rather than TH2 responses (Cheever, James, Scott and Sher).

Further evidence that T-cell subsets mediating immunity are different from those associated with immunopathology. Spleen and lymph node cells from mice infected with *S. mansoni* mounted strong IL-5 responses but weak responses of INF-gamma to worm and egg antigens. However, antigen stimulated T-cells from mice vaccinated against *S. mansoni* produced more INF-gamma than IL-5. These results support the observations noted above: i.e., immunity is associated primarily with TH-1 and immunopathology mainly with TH-2 responses (Pearce, Grzych and Sher).

MOLECULAR BIOLOGY OF SCHISTOSOMES

Several molecular approaches to improve activity of an anti-schistosomal vaccine. Paramyosin is a sub-tegumental component of schistosomes which has been shown to provide protection against infection when used as a vaccine. A near full length paramyosin clone provided by Dr. R. Pierce of the Pasteur Institute is being incorporated into *Salmonella* for use as an orally administered vaccine. To accomplish this the paramyosin clone must first be subcloned into an *E. coli* expression plasmid, and then, in turn, into another plasmid that will be expressed and integrated in *Salmonella*. The success of this molecular manipulation is currently being tested. Another approach consists of defining T-cell responsive epitopes on the paramyosin molecule by testing overlapping peptides that have been synthesized. An epitope has been defined consisting of 35 amino acids in the central portion of the molecule, that stimulates IFN-gamma production by T-cells of mice immunized with the native protein (Knight, Pearce, Sher and Houghten of Scripps).

Transposons in genome of schistosome may be involved in drug resistance. Last years's report indicated the detection of a new RFLP in the genome of drug resistant schistosomes as evidence for induction or mutation as the basis for resistance. The structure of the RFLP has been found to consist of a small ribosomal gene unit with a 0.65 kb insert in the non-transcribed spacer region. The RFLP is being sequenced to determine the nature of the insert, and its origin and source in the genome. These results indicate the existence of transposons in the genome of the schistosome, and suggest that induction of drug resistance may result from movement of mobile elements (Brindley, McCutchan, Sher and Lewis of BRI).

RIBOSOMAL RNA OF MALARIA PARASITES

More on ribosomal RNA genes during parasite development. The results reported last year have been carried further to define more precisely the conditions under which switching from one ribosomal gene to another occurs with *P. berghei*. For example, if sporozoites are irradiated, the ribosomal RNA found in sexual stages of the parasite continues to be present for a time and then disappears. Interestingly, the sexual stage type of ribosomal RNA already begins to be detected in gametocytes in the peripheral blood. Not surprisingly, the appearance of a ribonuclease which destroys the RNA of the previous developmental stage has been demonstrated when switching to a new stage occurs. Much more attention should probably be directed toward ribosomal RNA of parasites, not only for a better understanding of parasite development and physiology, but also as a target for new approaches in chemotherapy (McCutchan).

Use of oligonucleotide probes for rapid and sensitive diagnosis of malaria based on ribosomal RNA. Since ribosomal RNA is the most abundant cellular macromolecule, it is a logical target for detection in tiny amounts. A dot blot type of test of malarial parasite RNA has been developed that involves hybridization with radio-labelled oligonucleotides, followed by autoradiography. Suitable oligonucleotide probes have been prepared for all 4 human species of malarial parasites, and depending upon conditions of the test, the presence of 10 to 50 parasites can be detected. Sensitivity of the test can be increased by a longer hybridization period (up to overnight) or by raising the specific activity of the probes. Dried blood dots maintained stability of the mRNA for up to 4 days. This RNA detection method is much more sensitive than DNA hybridization methods and avoids technical problems of the complex PCR technique. Although need for the rRNA test in clinical diagnosis of malaria is not pressing and can await simplified modification, its use in epidemiological studies should be utilized as soon as possible (Waters and McCutchan).

MOLECULAR GENETICS OF MALARIA PARASITES

Chromosome mapping and drug resistance of P. falciparum. DNA probes for all of the P. falciparum nuclear chromosomes, as well as a mitochondrial chromosome are now available. RFLPs on 11 of the 14 nuclear chromosomes can be identified by these probes, and a search for RFLP markers to the remaining 3 nuclear and mitochondrial chromosome is being made. As reported last year, resistance to pyrimethamine in P. falciparum is linked to mutations in the dihydrofolate reductase (DHFR) enzyme. Now the actual locus in the enzyme, in terms of amino acid positions, has been identified where the mutation for resistance occurs. Yet, some of the pyrimethamine-resistant strains are sensitive to a different anti-fol drug, proguanil, and vice versa. Therefore, this helps explain why some pyrimethamine-resistant parasite strains may be effectively prophylaxed and treated with proguanil (Peterson and Wellems). Because only Ca^{++} channel blocking agent, verapamil, reverses chloroquine resistance in-vitro, it has been suggested that an ADP-dependent pump mechanism is responsible for the rapid efflux of chloroquine from resistant parasites. Some indirect evidence suggests that the gene responsible for drug resistance maps to chromosome 5 of the parasite. However, linkage data and tests with other probes on chromosome 5 fail to localize chloroquine resistance to chromosome 5. Instead, the data suggest that a single gene on another chromosome may be responsible for inheritance of the resistant phenotype (Walker and Wellems with Krogstad of Washington University).

Inherited factors affecting asexual proliferation of P. falciparum. Additional progeny from a second P. falciparum genetic cross were examined and found to confirm the presence of a subtelomeric region of chromosome 13 found in clones with the more rapid growth rate, as reported last year. The more rapid growth is due to increased efficiency of red cell invasion, not replication time. This locus on chromosome 13 also contains the gene for the histidine rich protein, HRP-III, but the possible role of the HRP-III locus in proliferation is not clear (Wellems and Dolan).

The genetic basis for another mechanism which can account for differences in invasion rates of P. falciparum is being explored. This mechanism involves dependence for sialic acid residues of glycophorin in the RBC membrane -- one of the molecules that plays a part in the receptor-mediated process of invasion. Parents of a genetic cross exhibit different invasion rates into red blood cells depending upon whether the RBCs have been treated with neuraminidase. Thus, it may be possible to identify the molecular basis of a non-sialic acid pathway of parasitic invasion (Dolan and Wellems).

MALARIAL ANTIGENS AS VACCINE CANDIDATES

Transmission blocking antigens of P. falciparum. The most promising sexual stage antigen (surface of ookinete), Pfs 25, has been cloned and can be expressed in bacterial and vaccinial expression systems. However, recombinant Pfs 25 is weakly antigenic, so ways are being sought to enhance antigenicity, such as production in mammalian cells with recombinant vaccinia virus, in yeast, or with baculovirus in insect cells. To map and define the most important B cell epitopes on Pfs 25, a series of 41 overlapping synthetic peptides were produced based upon the amino acid sequence of Pfs 25. Antisera to these peptides, as well as to recombinant Pfs 25 are being tested for transmission blocking activity (Kaslow, Quakyi and Miller of LPD; Isaacs and Moss of NIAID; Goldman of FDA; Houghton of Scripps and Snary of Wellcome Biotech of U.K.). Another gamete antigen, a 40 kDa surface protein, is being investigated for ability to block transmission (Rawlings and Kaslow; Coligan of NIAID).

Immunity to sporozoites and asexual parasites in murine systems. Immunity to sporozoites of P. yoelii via cytotoxic T-cells is under study in different strains of mice. The role of CD4+ T-cells has been investigated in immunity to asexual stage parasites; they are necessary in the case of P. vinckei but not with P. yoelii (Kumar, Weiss, Vinetz and Miller).

Further characterization of Duffy receptors for merozoite invasion make a receptor vaccine feasible. It has been known for some time that some components of Duffy blood group antigens serve as receptors for P. knowlesi and P. vivax malaria parasites. The 135 kD protein that binds to the Duffy receptor has been affinity purified and the corresponding gene from P. knowlesi is being characterized. Actually, several genes seem to be involved. A similar 135 kD protein from P. vivax that binds to Duffy positive red cells has been partially cloned. With P. vivax there seems to be a single gene for the receptor. As the molecular structure of these receptors become fully characterized, it should be possible to test the possibilities of making a vaccine to raise antibodies to block merozoite invasion (Miller, Adams, Hudson and Fang, Klotz and Haynes of WRAIR and Dalton of Univ. of Dublin in Ireland).

GENETICS AND PHYSIOLOGY OF ANOPHELINE VECTORS

Mosquito genes that might be manipulated for vector control. Physiologic differences in regard to malaria parasite development in susceptible as compared to refractory mosquitoes provide potential targets by which transmission might be controlled. Mosquitoes refractory to parasite development encapsulate the oocysts by a process of serial activation of enzymes that ultimately deposit melanin pigments around the oocyst. Several phenoloxidase proteins which serve as substrates of the activating enzymes are being purified. The gene for one of the proteins has been cloned and is also present in Drosophila. Several RFLPs of this gene have been identified in refractory and susceptible lines of A. gambiae so it should be possible to investigate the inheritance of these products. The encapsulation response to ookinetes of the avian malaria parasite, P. gallinaceum, in A. gambiae is also being studied (K. P. Sieber, Huber, Gwadz and Miller).

Transformation via P vector turns out to have disappointingly low frequency. Although one stable transformation out of 2200 A. gambiae injected eggs was obtained, no further examples of stable expression of injected foreign DNA were found in another 5000 eggs injected. Other methods of transformation which might be more efficient are being explored (Miller, Sakai and Gwadz).

Can vitellogenin genes be exploited to influence parasite development in the mosquito? Vitellogenin is a material produced for making eggs after a blood meal by the female mosquito. The genes controlling production of vitellogenin are being characterized (i.e. sequenced, identification of promotor and regulatory regions) with the idea that they might ultimately be manipulated to influence development of the malaria parasite (Romans).

IMMUNOREGULATION OF B- AND T-CELL RESPONSES

IgE responses. There is increasing evidence, much of it from studies in the mouse, that lymphokines produced by different subsets of T-cells regulate the production of different types of antibodies. As reported last year, T-cell clones from patients with parasitic infections (filariasis), as well as a non-parasitic infection (Hyper-IgE syndrome), and both states characterized by high serum IgE levels, were shown to be making large amounts of IgE binding factor. Further characterization of some of these T-cell clones has shown them to be making IL-4, IL-5 or IL-6; thus, they appear to be a human equivalent of the mouse TH2 subset of T-cells. Studies with recombinant lymphokines showed that INF-gamma down-regulated B-cell IgE responses in-vitro in patients with hyper IgE states. In contrast, IL-4 had an opposing effect on human lymphocytes causing marked enhancement of IgE responses. Therefore, INF-gamma has been given to patients with the hyper-IgE syndrome. Suppression of IgE synthesis by their B-cells has been demonstrated in-vitro in all patients tested, and serum IgE levels have been significantly lowered in about half of the patients studied (King, Malech, Gallin and Nutman).

Estimates of precursor frequency of IgE producing B cells have been developed with an ELISPOT assay, and will be tried with in-situ hybridization as well. Already results indicate that individuals with elevated IgE levels have 10 to 100-fold more B-cells committed to IgE production than do normal individuals. The influence of lymphokines for induction of parasite-specific IgE is also being examined in in-vitro model systems driven by antigen (King, Ottesen and Nutman).

IgG subclass responses. The regulation of serum protein IgG subclasses was also examined in-vitro and in-vivo. The cytokines IL-4 and INF-gamma were found to regulate IgG1, IgG3 and IgG4 without having an effect on IgG2. It appears that INF-gamma acts upon IgG4 production in the same manner as it does for IgE (King, Ottesen and Nutman).

T-cell responses. The lack of T-cell responses in microfilaremic patients with lymphatic filariasis was reported last year to be due to immunosuppressive properties of phosphocholine containing filarial antigens found in the circulation of these patients. In contrast, T-cell hyperreactivity to parasite antigens has been found in some patients with another filarial disease, loiasis. By T-cell Western blotting 6 different antigen fractions have been identified which cause T-cell proliferation. The nature of these antigens is under investigation (Klion and Nutman).

The role of IL-5 in the control of eosinophilia of parasitic infections is being studied. Optimum conditions for induction of IL-5 by detection of mRNA for IL-5 and actual assay of the material have been developed. The lymphocytes of patients with eosinophilia contain more IL-5 than patients without eosinophilia, and IL-5 production can be induced by parasite antigen in those previously sensitized (Limaye, Ottesen and Nutman).

IMMUNODIAGNOSIS AND TREATMENT OF FILARIASIS

Molecular definition of filarial antigens. As reported last year, increasing use is being made of antigens produced from cloned gene products to examine antibody responses of patients with different filarial infections. Particular effort is being made to demonstrate antibody responses that are completely specific to a given parasite, and, if possible,, to a given clinical entity caused by the parasite. For example, a 16 kD recombinant antigen obtained from a cDNA library of adult worm Onchocerca volvulus, appears to be very specific and even detects antibodies in the pre-patent period -- i.e., early stage before microfilariae are produced (Lobos, Nutman and Ottesen). Interestingly, the recombinant antigen can be readily purified with the use of Factor X from human serum. Bronchoalveolar lavage (BAL) fluid from patients with acute tropical pulmonary eosinophilia was shown by immunoblotting to contain antibody, presumably produced locally in the lung, to a distinct subset of antigens (Nutman and collaborators in India).

Phosphocholine and IgG4 antibody response. Phosphocholine (PC) is a prominent antigenic component of many helminthic and protozoan parasites, thereby often being responsible for immunologic cross-reactivity or "non-specific" reactions in immunologic tests using crude parasite antigens. Since humans with chronic helminthic infections generally have been found to have high titers of IgG4 antibodies, and since anti-PC responses in humans are restricted in the sense that no anti-PC IgG4 responses are made, antibody tests based on detection of IgG4 antibodies is particularly useful (Lal, Ottesen, Hussein).

Ivermectin treatment of lymphatic filariasis. Several trials have now shown that the efficacy of single oral dose Ivermectin at as low dose as 20 mcg/kg was equivalent to that of diethylcarbamazine (DEC) for 2 weeks when assessed over the short term (3 mos.). However, microfilarial rates were up again more quickly in the Ivermectin (30%) than in the DEC treated group (15%). Current trials have been planned and initiated in both South India and in Brazil to determine the lowest effective dose of Ivermectin (Ottesen with collaborators in India and in Brazil).

MOLECULAR BIOLOGY OF GIARDIA AND AMEBAE

Further molecular analysis of Giardia. Last year's work concentrated heavily upon the 170 kD variant surface antigen, its presence in many isolates of the parasite, and patterns of its appearance and disappearance. Sequence analysis of the 170 kD antigen showed an unusually rich cysteine content. Variant parasites which are direct progeny of the 170 kD containing strain, which no longer contain the 170 kD antigen, show the presence of a cysteine-rich peptide, suggesting functionally relatedness (Nash, Mowatt and Adam).

A gene encoding for a 29.4 kD protein was found to have a sequence consistent with one of the giardins previously described. In contrast to the 170 kD antigen the 29.4 kD protein seems to be a conserved gene product. Antisera to the fusion protein react with the axostyle and sucking disk of the parasite (Mowatt and Nash).

Binding of tricyclic anti-depressant drugs to Giardia. Although the tricyclic anti-depressant drugs such as imipramine and chlorimipramine are not used clinically for treatment of giardiasis, they have a novel mechanism of action and differential toxicity against the parasite. Therefore, it seems worthwhile to study the kinetics of binding of different radiolabelled analogues of this class of drugs. Although chlorimipramine is 3-fold more toxic to the parasite than the parent compound imipramine, uptake of the chloro analog was only 30 per cent more. The drugs show both specific and non-specific binding to the parasite, as judged by competitive inhibition with unlabelled analogs of imipramine. Extending the kinetic data to Scatchard plots to ascertain quantitative binding affinities is planned (Weinbach and Levenbook).

Ribosomal DNA of amebae. As reported last year, ribosomal RNA genes of E. histolytica were shown to be located on extrachromosomal DNA circles. With a yeast plasmid vector and restriction enzyme analysis, an origin of replication has been tentatively located on a 6.8 kb Eco R1 fragment. Restriction enzyme analysis of ribosomal DNA from 4 clones of one strain of E. histolytica showed two different patterns. One pattern appeared to result from co-cultivation with T. cruzi, which is often used as an associated organism when amebae are being axenized. This may represent the first evidence for molecular alteration of amebae when grown with another protozoan (S. Bhattacharya and Diamond).

E. histolytica antigens defined by monoclonal antibodies (Mabs). An antigen, present in the cytoplasmic compartment of E. histolytica, with a molecular weight of 28 kD, was found to be released from the cell during growth. It is also recognized by serum from infected patients (A. Bhattacharya and Diamond). The specificity of two DNA probes (P145 and B133) developed in Israel for distinguishing pathogenic vs. non-pathogenic strains of E. histolytica, according to their isoenzyme profiles were tested in a double blind study on 19 different amebae. The pathogenic probe, P145, recognized only pathogenic E. histolytica, while the non-pathogenic probe, B133, recognized only non-pathogenic isolates (Diamond and Mirelmann of Israel).

LEISHMANIAL BIOCHEMISTRY AND CELL BIOLOGY

Secretory acid phosphatase (SAcP) of Leishmania made in the golgi compartment. Cumulative results from metabolic labeling studies using various inhibitors as well as EM have demonstrated that the leishmanial SAcP is processed in the golgi compartment of the organism (Bates, Bonsch-Hermes and Dwyer).

Biosynthesis of SAcP by amastigotes. With metabolic labeling and immunoprecipitation it was shown that L. donovani amastigotes actively synthesized SAcP within infected human macrophages in-vitro. Moreover, the amastigote SAcP was also detected in culture supernates indicating that the parasite enzyme was being released into the extracellular space. When SAcPs of 18 different reference strains of Leishmania, representing 7 different species, were compared, distinct electrophoretic profiles between species were demonstrable (Doyle and Dwyer).

Further analysis of SAcPs of Leishmania. SAcP was tested for its sensitivity to many different proteases and found to be resistant. In fact, even though SDS-PAGE analysis showed that the enzyme was cleaved by proteases, functional activity remained. Attempts are underway to clone and sequence SAcP genes (Mallinson and Dwyer).

Growth of cell-free "amastigotes" of Leishmania. A standard L. donovani strain has been maintained by serial passage for 18 months in 100% heat inactivated fetal calf serum (FCS) at 37°C. It is morphologically similar to the intra-cellular amastigote form and has a doubling time identical to that of the organism grown at 26°C in either 100% FCS or in other liquid media. By flow cytometry these "amastigotes" have a similar DNA pattern to promastigotes grown at 26°C (Doyle and Dwyer).

Lipophosphoglycan (LPG) of L. major undergoes a change in infective (metacyclic) form. Previous reports have described the finding that L. major organisms in culture and in the sandfly vector during the log phase of growth are essentially non-infective, and become infective after reaching the stationary growth phase. This change is also accompanied by resistance to complement-mediated killing and loss of agglutination by lectin peanut agglutinin. Carbohydrate analysis of extracted metacyclic LPG shows a doubling in phosphorylated saccharide units and a thickening in the LPG coat and different structure by freeze-fracture EM. This modification of the surface LPG promotes complement resistance, but continues to activate complement, depositing C3b on the parasite surface and increasing attachment to and uptake by mouse and human macrophages. Serum opsonized metacyclics actually survive better within macrophages than non-serum incubated parasites (Sacks, da Silva, Puentes, Pimenta of NCI and Turco of Univ. of Kentucky).

T. CRUZI AND CHAGAS' DISEASE

Molecular and chromosomal analysis of T. cruzi. Epimastigotes and culture derived trypomastigotes of T. cruzi grown at different temperatures show slightly but definitely different amounts of DNA. A mutant clone of T. cruzi has been detected that arose spontaneously and contains much more DNA per cell than other clones. The great range in molecular weights of T. cruzi chromosomes has made identification of individual chromosomes by pulse-gel electrophoresis very difficult (Dvorak and Engel).

A microcomputer-based flow cytometry data collection and analysis system. Over the last 3 years a microcomputer based system has been developed which overcomes many limitations of presently available instrumentation. Application for a patent has been made because the system may be useful for a broad range of biomedically important problems. The most novel and useful software feature consists of a modified Box-Cox equation for dynamic data transformation (Dvorak and Banks of OSD, NIAID).

Anti-trypanosomal factor (ATF) from Pseudomonas. Attempts continue to purify and elucidate the chemical structure of ATF (Mercado and collaborators).

IMMUNOLOGY OF STRONGYLOIDIASIS

Further characterization of larval protease. Several metallo-protease inhibitors reduced penetration of L3s through rat skin. A monoclonal antibody against the protease has been made and purification of the protease is underway (Brindley, Gam, Neva and McKerrow of U Calif.).

Strongyloidiasis in human retrovirus infections. Several collaborative studies are underway to examine the importance of strongyloidiasis as an opportunistic infection in patients with AIDS and with HTLV-1. Preliminary findings suggest it is not a big problem in AIDS patients, but may be in some patients with HTLV-1 (Neva, Blattner of NCI; collaborators in Jamaica; Brown and Perreins of Project SIDA in Zaire).

UNIT ON MICROBIAL PATHOGENESIS

FACTORS AFFECTING PARASITE ATTACHMENT TO CELLS

Effect of C1q on T. cruzi. The enhancing effect of one of the complement components of serum, C1q, on internalization of T. cruzi by human monocytes and macrophages was reported last year. Now, the same enhancement was also shown for both attachment and internalization of T. cruzi into fibroblasts which are nonphagocytic cells (Joiner).

Effect of mannose binding protein (MBP) on Salmonella. MBP, a component of normal human serum, binds to bacterial cell walls rich in mannose. MBP also enhances deposition of C3 on the bacterial cell wall via the alternative complement pathway. Thus, in the presence of MBP, bacteria rich in mannose may be lysed in non-immune serum, or opsonized for ingestion by neutrophils or monocytes (Schweinle and Joiner).

Laminin and Toxoplasma. Laminin, a basement membrane protein, augments attachment and entry of tachyzoites of T. gondii into macrophages and fibroblasts (Fuhrman and Joiner).

INTRACELLULAR COMPARTMENTALIZATION OF MICROORGANISMS

Salmonella in neutrophil phagosomes. Using several different methods of analysis (immuno-EM, immunofluorescence, and immunoblotting) neutrophil specific granule constituents were found to be preferentially incorporated into phagosomes containing IgG coated Salmonella. However, the same constituents were much less prominent in phagosomes containing C3 coated bacteria. Cell surface membrane receptors, such as CR1, CR3, and Fc4111, were indiscriminately incorporated into the phagosome, indicating selectivity in neutrophil specific granule fusion (Schweinle, Joiner and Rotrosen of LCI).

T. cruzi in J774 host cells. The technique that has been used to study the membrane composition of phagolysosomes containing yeast in J774 cells was applied to T. cruzi. Using both intra-cellular iodination and immunofluorescence it was shown that non-infective epimastigotes were internalized into vacuoles which contain the complement receptor, CR3, whereas infective metacyclic trypomastigotes reside in CR3 negative vacuoles. Both compartments contain lysosome-specific glycoproteins, indicating that the parasitophorous vacuole has fused with lysosomes. In the case of trypomastigotes, they rupture out of the vacuole in a matter of hours and begin to replicate (Joiner and Mellman at Yale).

Toxoplasma in Chinese hamster ovary (CHO) cells. Toxoplasma organisms which enter CHO cells reside in compartments which neither acidify or fuse with lysosomes. Antibody coated or heat killed tachyzoites entering CHO cells transfected with the murine FcR11 receptor reside in compartments which acidify and have fused with lysosomes. The transfection with murine Fc receptors (FcR) was necessary to avoid a respiratory burst during parasite entry (Joiner and Mellman at Yale).

These results illustrate 3 different forms of selectivity in incorporation or exclusion of membrane receptors and/or granule constituents into phagosomes.

Possible molecular transformation of T. cruzi. Methods are being developed to examine molecular mechanisms controlling gene expression in T. cruzi. Toward this end transcriptional activators for yeast (GCN4) and mammalian cells (e.g., c-fos and c-myc) were tested for possible homology with T. cruzi genes. The c-fos proto-oncogene was found to hybridize to T. cruzi DNA. A method of growing T. cruzi on solid media, as colonies originating from single organisms, was developed to detect foreign DNA by colony hybridization. T. cruzi epimastigotes were successfully transfected with plasmid DNA by fusion of liposomes containing DNA with the parasite, a process known as lipofection (Ogden and Joiner).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00094-30 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Entamoeba histolytica and related Entamoeba: Taxonomy, Virulence, Nutrition.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.S. Diamond	Section Head	LPD, NIAID
Others:	A. Bhattacharya	Guest Worker	LPD, NIAID
	S. Bhattacharya	Guest Worker	LPD, NIAID
	E.C. Weinbach	Section Head	LPD, NIAID

COOPERATING UNITS (if any)

Department of Biophysics, Weizmann Institute of Science (David Mirelman), American Type Culture Collection (P.M. Daggett, Tom Nerad), Medical Research Center, VA (A.T. Soldo).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasite Growth & Differentiation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have shown that rRNA genes of Entamoeba histolytica reside on extrachromosomal DNA circles. Restriction enzyme maps of these genes can be used in distinguishing between isolates and species of Entamoeba. New studies using a yeast plasmid vector, and restriction enzyme analysis provided evidence the circles are self-replicating, and an origin of replication has been located tentatively on a 6.8kb EcoRI fragment. Hypervariable polymorphic loci have been found within the circles. Two have been studied in cloned populations of the same amebal strain. One locus appeared to be an innate variable, the other to be generated as a consequence of cocultivation with the pathogenic protozoan, Trypanosoma cruzi, which is often used to support amebal growth prior to axenization. This may be the first evidence at the molecular level of the alteration of E. histolytica grown in association with other protozoa.

A dominant antigen recognized by serum from humans infected with E. histolytica has been characterized as having a molecular weight of 28 kD, is released from the cell during in vitro growth, is not associated with any membrane, and is located in the cytoplasmic compartment.

Study of an antigen recognized by the monoclonal antibody, 2D7.10, specific for E. histolytica, provided tentative evidence that the epitope recognized by the antibody is a carbohydrate.

The specificity of two DNA probes developed at the Weizmann Institute of Science to distinguish strains of E. histolytica classified on the basis of their isoenzyme profiles as pathogenic or nonpathogenic was confirmed through a double-blind study entailing examination of several strains of E. histolytica, related species of Entamoeba, and unrelated enteric protozoa of the gut.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00097-31 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	H.M. Fales	Chief, Laboratory of Chemistry	IR, CH, NHLBI

COOPERATING UNITS *(if any)*

Waters Life Science Application Laboratory, Fairfax, VA
(M. P. Strickler)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the purification of an anti-trypanosomal factor from the bacterial species, Pseudomonas fluorescens, were continued. Countercurrent chromatography (CCC) with the Ito multilayer coiled column and a high pressure chromatography system (HPLC) consisting of both preparative and analytical carbohydrate analysis columns were used. Although the lytic activity of this compound was enhanced significantly with this technique, samples subjected to NMR analysis and mass spectroscopy revealed a degree of contamination which prevented a clear elucidation of the chemical structure. In an attempt to remove the contaminating components reversed phase HPLC of ethanolic extracts without CCC is being used and lytic fractions are being collected for further analyses. The determination of chemical structure is essential before studies on synthesis are initiated and pharmacological and toxicological properties are examined employing infections with Trypanosoma cruzi.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00098-33 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continued studies of the uptake of the tricyclic drugs imipramine and chlorimipramine by trophozoites of Giardia lamblia showed that these compounds, although highly lipophilic, are bound to protein sites of the parasite. Delipidized trophozoites still bound substantial amounts of the drugs, whereas treatment of G. lamblia with protein denaturing agents released 90% of the bound drugs. The tricyclic compounds exhibited both non-specific and specific binding to the parasites. Imipramine and chlorimipramine released protein(s) from G. lamblia, the chloro analog being 2-3 fold more effective. We found a correlation between those drugs that are weak inhibitors of parasite growth and their ability to release protein(s); this may be the basis of their differential toxicities. Mammalian studies established that of the halogenated analogs 3-bromoimipramine was the most effective uncoupler of ATP synthesis in mammalian liver mitochondria.

PHS 6040 (Rev 1/84)

GPO 914-918

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00099-19 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Biophysical Parasitology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Branch, DRS; Laboratory of Applied Studies, DCRT; FIOCRUZ, Rio de Janeiro, Brasil

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project is concerned with (a) analyses of the genetic diversity of Trypanosoma cruzi and its implications to the epidemiology, course and diagnosis of Chagas' disease, (b) the development of high resolution flow cytometry instrumentation for biomedical research and, (c) the utilization of flow cytometry and low light level video microscopy for the analyses of infectious agents. We have established that interdevelopmental stage differences in total DNA exists in T. cruzi. Hybridization analyses with DNA probes indicate that T. cruzi exhibits restriction fragment length polymorphism (RFLP) and preliminary data indicate this may be due to amplified DNA or multiple repeated sequences. Although definitely a low frequency occupance, mutants of T. cruzi can arise spontaneously which may explain both the appearance and maintenance of T. cruzi diversity in nature.

Others continued

J.J. Bailey	Senior Investigator	LAS, DCRT
S. Goldenberg	Professor	FIOCRUZ, Brasil

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00102-15 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F.A. Neva	Chief	LPD, NIAID
Others:	D. Sacks	Research Microbiologist	LPD, NIAID
	P. Melby	NRSA Fellow	LPD, NIAID

COOPERATING UNITS *(if any)* Inst de Salubridad y Enfermadades Trop, Mexico City (O. Velasco); U Md, Ctr for Vaccine Dvlpmt (S Savarino, S Giannini); Min of Hlth, Tegucigalpa, Honduras (C Ponce); Dept Biol, Youngstown U, Ohio (R Kreutzer); Yale U Sch of Publ Hlth, Dept Epidemiology, New Haven, CT (D McMahon-Pratt); ER Squibb and Sons, Inc., Princeton, NJ (AL Lentnek).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has continued to focus upon different clinical forms of leishmanial infection in humans, the immune response to them, and characteristics of the causative parasites.

In the past year five new cases of cutaneous leishmaniasis were studied at the Clinical Center. T-cell lines as well as several clones were developed from biopsies of skin lesions. Clones of both CD4 and CD8 phenotypes have been identified and will be used to study their responses to specific and non-specific antigenic stimuli.

Major effort was given to collaborative studies with Dr. Carlos Ponce of Honduras of the unusual form of cutaneous leishmaniasis caused by L. donovani chagasi, the species that normally causes the visceral form of disease. It now appears that the atypical cutaneous disease is relatively common in Honduras, but the pathogenesis of this form of leishmaniasis is not understood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00108-18 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Studies on the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	L.H. Miller	Head, Malaria Section	LPD, NIAID
	S. Kumar	Visiting Fellow	LPD, NIAID
Others:	T. McCutchan	Senior Scientist	LPD, NIAID
	W. Weiss	Guest Worker	LPD, NIAID
	Joe Vinetz	Hughes Medical Student	LPD, NIAID

COOPERATING UNITS *(if any)*

W.L. Maloy, Senior Scientist, LIC, NIAID, J.A. Berzofsky, Senior Scientist, Metabolism Branch, NCI; B. Greenwood, British MRC, The Gambia, West Africa; R. Houghten, M. So, Scripps Clinic, LaJolla, CA; M.F. Good, QIMR, Brisbane, Australia.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Circumsporozoite (CS) proteins have been shown to act as the protective antigens capable of inducing sporozoite-specific immunity in several species of malaria. The gene coding for this antigen in Plasmodium falciparum has been isolated, cloned, and incorporated into E. coli. The recombinant protein induces antibodies in mice which react with sporozoites and blocks liver invasion in vitro.

T cells are critical for malaria immunity, not only because they serve as helper cells for an antibody response, but because they also have an important role as effector cells. The level of the immune response to a given immunogen is determined by Immune Response (Ir) genes and tolerance. We have studied the interaction of the malaria parasite with the host immune system and we have found evidence that the parasite has lessened the level of the immune response against it by host mimicry (and resultant immunological tolerance) and by limitation and variation within T sites (i.e. through Ir gene control). The studies cover sporozoites and asexual erythrocytic parasites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00161-12 LPD

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	F.A. Neva	Chief	LPD, NIAID
	M. Ballard	Medical Staff Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID
	M.M. Mowatt	Staff Fellow	LPD, NIAID
	B. Gottstein	Visiting Scientist	LPD, NIAID

COOPERATING UNITS *(if any)*
M.M. Levine, D.A. Herrington, G.A. Losonsky, University of Maryland

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.3	2.8	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biological importance of antigenic variation in Giardia will depend in part on the size, nature, and rate of change of the variant surface antigens. The presence of epitopes on the surfaces of trophozoites from 27 isolates and numerous clones were determined. The presence of defined epitopes was dependent on the isolate. Three epitopes (defined with Mabs 6E7, 5C1, and 3F6) tended to appear in the same culture together or singly and another epitope (G10/4) was found only in two isolates, but never in the presence of any of the other three epitopes. A number of isolates failed to react with any Mabs. Clones derived from isolates with a certain pattern of reactivity were able to generate small numbers of trophozoites with the same pattern of reactivity. On the average this occurred once every 14 generations. Double labeling studies showed only one epitope on the surface of reactive trophozoites. Analysis of the surfaces of Mab 6E7 reactive clones derived from isolates CAT-1, Be-2 and G3M revealed Mab 6E7 reacted with antigens of different molecular weights. Giardia isolates differ in their ability to generate certain epitopes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00162-13 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD,NIAID
Others:	P.S. Doyle	Visiting Fellow, FIC	LPD,NIAID
	G.O. Gbenle	Intnatl Res Exchg Fellow, FIC	LPD,NIAID
	D.J. Mallinson	Visiting Fellow, FIC	LPD,NIAID
	C. McCarthy-Burke	Biologist	LPD,NIAID

COOPERATING UNITS *(if any)* Dept Zool, Univ Glasgow (P Bates); Dept Immun Infect Dis, Johns Hopkins Univ (M Gottlieb); Inst Med Parasit, Univ Bonn (I Bonsch-Hermes); Dept Biochem, Univ Victoria (R Olafson); Dept Biochem, Univ Kentucky (S Turco); Dept Biol, Technion-Israel Inst Tech (D Zilberstein).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology & Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
4.0	3.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cell biology of Leishmania is investigated as a model of both intra- and extracellular parasitism. Emphasis is placed on characterizing the biochemical and physiological functions of its surface membrane (SM) and secreted components toward defining their roles in parasite survival and development.

The SM proline-transport protein of L. donovani promastigotes was identified using an anti-idiotype antibody (α-ID). The α-ID inhibited proline uptake by intact LPG(-) promastigotes and immunoprecipitated two SM proteins of ~57 and 52 kDa. The α-ID also identified several clones expressing the proline transporter gene in a lambda library. Additional physical and immunochemical properties of the SM acid phosphatase were delineated and the enzyme purified. A new SM purine-salvage enzyme, of possible hybrid gene origin, was identified and partially purified. Golgi-mediated biosynthetic processing of the secretory acid phosphatase (SAcP) was defined and localized. The SAcP was shown to possess the unique $[-PO_4\text{-Gal-Man-}]_n$ repetitive epitope in common with the SM lipophosphoglycan. Amastigotes were shown to actively synthesize and secrete a distinct isoform of SAcP. The SAcP's of virtually all leishmanial species were shown to possess conserved cross-reactive antigenic epitopes. Further, patients with all forms of leishmaniasis were found to have antibodies against this enzyme. The active/catalytic site of native SAcP was shown to be highly resistant to proteolysis, a property relevant to its biological function. A simple cultivation method was devised for the continuous in vitro growth and serial propagation of "amastigote-like" forms (AmF) of L. donovani at 37°C. The AmF express a unique set of ~63-70 kDa SM proteins and secrete an isoform of SAcP typical of amastigotes. The availability of such forms should greatly facilitate studies of parasite gene regulated differentiation and development.

The current results are of relevance toward the development of improved diagnostic, chemotherapeutic and immunoprophylactic agents.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00197-10 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Immunoregulation and immune recognition in filariasis and non-filarial diseases.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T.B. Nutman	Senior Investigator	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	D. Freedman	Medical Staff Fellow	LPD, NIAID
	C.L. King	Medical Staff Fellow	LPD, NIAID
	A. Limaye	Howard Hughes Research Scholar	LPD, NIAID
	H.L. Malech	Senior Investigator	LCI, NIAID
	J.I. Gallin	Director	IRP, NIAID

COOPERATING UNITS (if any)

Department of Allergy and Clinical Immunology, University of Montreal, Montreal, Canada (G. Delespesse); Department of Onchocerciasis, SNEM, Guatemala City, Guatemala (G. Zea-Flores); Uniformed Services Univ of the Health Sciences (R. Lal).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.7	2.0	0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariasis and other disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy at the level of antibody (B cell) and lymphokine (T cell) responsiveness. Further, similar T cell anergy has been found among patients with active onchocerciasis. The influence of genetic factors, antigen composition, and specific antifilarial chemotherapy on this anergy has begun using molecular biological tools and defined antigens.

In vitro models of parasite-antigen driven antibody production as well as parasite-specific and HTLV-I transformed T cell clones have been developed to understand in more detail those mechanisms regulating antibody production (particularly IgG and IgE) in filarial and non-filarial diseases. Recombinant lymphokines have provided additional tools for defining the mediators involved in this regulation.

Qualitative analysis of filaria-specific IgE and IgG in loiasis, lymphatic filariasis, and onchocerciasis have indicated patterns of antigen recognition which differ among groups of patients with different clinical manifestations of filariasis. Using these techniques, possible vaccine targets have been identified for use in onchocerciasis. Filarial species-specific antigens recognized by human monoclonal antibodies derived from EBV-transformed patient B cells have also been identified and characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00208-09 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.F. McCutchan	Microbiologist	LPD, NIAID
Others:	G. McConkey	IRTA Fellow	LPD, NIAID
	K. Vernick	Technician	LPD, NIAID
	A. Waters	Visiting Associate	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been characterizing surface antigen genes from Plasmodium falciparum in the hope of contributing to the construction of an anti-malaria vaccine. One interesting aspect of this study has focused on the variation in the circumsporozoite gene of P. falciparum. Results indicate that this variation is likely to be biologically selected because of interaction of the parasite surface molecule with host T-cells.

The ribosomal RNA of different malarial parasites has been another focus of study. Information from these studies has important ramifications for the development of malaria diagnostics, the understanding of evolutionary relationships among the different malarial species and the understanding of control of the parasites developmental cycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00240-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	D. Kaslow	Medical Staff Fellow	LPD, NIAID
	L.H. Miller	Head, Malaria Section	LPD, NIAID
Others:	C. Syin	Guest Worker	LPD, NIAID
	I. Quakyi	Guest Worker	LPD, NIAID
	D. Rawlings	Guest Worker	LPD, NIAID
	D. Keister	Biologist	LPD, NIAID
	J. Coligan	Branch Chief	BRB, NIAID

Others continued below.

COOPERATING UNITS *(if any)* Papua New Guinea Inst Med Res (P Graves); Hazelton Labs, Vienna VA (J Rener); U Edinburgh, Scotland (R Carter); Sch of Pub Hlth, J Hopkins U, Baltimore MD (N Kumar); Cetus Corp, Emeryville CA (J Nunberg); Scripps Clin Res Fnd, La Jolla CA (R Houghton); QIMR Brisbane, Australia (M Good); cooperating units continued below

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
4.6	3.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Proteins on the surface of sexual stage malaria parasites are targets of antibodies that block infectivity of gametocytes to mosquitoes. All but one of these proteins were shown to induce limited immune responsiveness in MHC disparate congenic mice strains. Limited T cell recognition of a 40 kDa surface protein suggests that this protein has been under immune selection and therefore may be involved in transmission blocking immunity. This previously undescribed protein is being investigated. Pfs25 was found to be immunogenic in all of the mice strains tested. We have cloned the gene coding for Pfs25, as well as the analogous gene in Plasmodium gallinaceum, Pgs25, and now can express and raise antisera to recombinant Pfs25 protein produced in bacterial and in vaccinial expression systems. To map and define the B cell epitopes that may be crucial in developing a peptide vaccine for Pfs25, antisera to a series of 41 overlapping peptides synthesized on the basis of deduced amino acid sequence of Pfs25 have been produced. Currently, antisera to these peptides and to recombinant Pfs25 are being tested for transmission blocking activity.

Others cont.

S. Isaacs	Medical Staff Fellow	LVD, NIAID
B. Moss	Lab Chief	LVD, NIAID
J. Bersofsky	Head, MIVRS SECTION	MET, NCIT

Cooperating Units cont.

Wellcome Biotech, Kent, England (D Snary); Bureau of Biol, FDA, Beth MD (N Goldman); CDC, Atlanta GA (B Collins).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00241-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.H. Miller	Section Head	LPD, NIAID
Others:	J. Adams	Staff Fellow	LPD, NIAID
	D. Hudson	Microbiologist	LPD, NIAID
	T. Wellems	Sr. Staff Fellow	LPD, NIAID
	D. Kaslow	Staff Fellow	LPD, NIAID
	X. Fang	Visiting Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*

WRAIR, Washington, DC (F. Klotz and J.D. Haynes); Hazelton Laboratories, Vienna, VA (J. Rener); Case Western Reserve Univ., Cleveland, OH (M. Aikawa); University of Dublin (J. Dalton)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	3.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage against which immunity may work to block invasion. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying P. knowlesi, P. vivax and P. falciparum receptors for attachment to monkey and human erythrocytes. We are studying one antigen on the merozoite surface that undergoes antigenic variation to understand the molecular basis for this variation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00244-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Studies on the Developmental Adaptation of Trypanosoma cruzi to the Vertebrate Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	S. Heath	Visiting Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

In this project we have been studying developmental adaptations of T. cruzi to the vertebrate host and, in particular, the molecular basis of adaptive changes occurring during the differentiation of epimastigotes (vector stage) to metacyclic trypomastigotes (infective stage).

A metacyclic stage specific cDNA was shown to be inducible by cAMP in epimastigotes. Genomic sequencing of a 5 kb fragment containing the gene indicates that it may be tandemly repeated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00246-07 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Molecular Studies of the Genome and Surface of Schistosoma mansoni

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E.J. Pearce	Visiting Associate	LPD, NIAID
	M. Knight	Special Volunteer	LPD, NIAID
	P. Brindley	Special Volunteer	LPD, NIAID
	T. McCutchan	Senior Investigator	LPD, NIAID

COOPERATING UNITS *(if any)*

Biomedical Research Institute, Rockville, MD (F. Lewis); Scripps Research Foundation, LaJolla, CA (R. Houghten); Fiocruz, Belo Horizonte, Brazil (R. Correa-Oliveira)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.1	2.6 0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The aim of this project is to characterize schistosome surface molecules relevant to immunity, to analyze the genome of the parasite in relation to specific biological properties of the organism and to clone important schistosome immunogens.

A. Molecular immunology of paramyosin. A near full length clone of paramyosin was subcloned into plasmids in preparation for expression in Salmonella. Overlapping peptides spanning the paramyosin sequence were synthesized and mapped for their T cell reactivity.

B. Selective recognition of paramyosin after chemotherapy. In a field study, antibodies against paramyosin were shown to increase dramatically and selectively after chemotherapy and were maintained in high levels in individuals displaying complete drug cure.

C. Structure of an RFLP associated with drug resistance. An RFLP associated with drug resistant schistosomes was shown to result from the insertion of a mobile element into a ribosomal gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00248-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R.W. Gwadz	Senior Scientist	LPD, NIAID
	L.H. Miller	Section Head	LPD, NIAID
	R.K. Sakai	Expert	LPD, NIAID
Others:	P.A.J. Romans	Visiting Fellow	LPD, NIAID
	K.-P. Sieber	Staff Fellow	LPD, NIAID
	M. Huber	Visiting Fellow	LPD, NIAID
	C. Graziosi	Guest Worker	LPD, NIAID

Others continued below.

COOPERATING UNITS (if any)

Dept of Entomology, U of Maryland, College Park, MD (Dr M Ma); Inst of Parasitology, U of Rome (Dr M Coluzzi); Inst of Pathology, Case Western Reserve School of Med (Dr M Aikawa); Ecole Nationale de Medecine, Bamako, Mali (Dr YT Toure); WRAIR (KL Sim)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.2	5.2	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The molecular biology of anopheline mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Systems for cloning and transposing genes into mosquito germ lines are being developed. Factors which render mosquitoes refractory to malarial infection, with special emphasis on mechanisms which encapsulate developing parasites, are being defined. Linkage of refractory genes to genes regulating blood-meal associated physiological events, e.g., vitellogenin synthesis, will be used to enhance refractory responses.

Additional parasiticidal mechanisms from within and outside of mosquitoes are being sought with special emphasis on the evaluation of magainins, cecropins and defensins, their physiological effects on in vivo parasite development and the molecular biology of the genes coding for their production.

The ability to identify, clone and transpose genes which regulate refractory mechanisms should permit the development of mosquito lines incapable of transmitting malaria for use in malaria control schemes.

Other Investigators cont.

Others:	H.G. Coon	Senior Investigator	LG, NCI
	P. Kantoff	Senior Investigator	LMH, NHLBI
	K. Mizuuchi	Senior Investigator	LMB, NIDDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00251-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E.J. Pearce	Visiting Associate	LPD, NIAID
	S.L. James	Program Officer	MIDP, IRP, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID
	P. Scott	Expert	LPD, NIAID
	J.M. Grzych	Special Volunteer	LPD, NIAID

COOPERATING UNITS *(if any)*

DNAX Research Institute, Palo Alto, CA (R. Coffman).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The aim of this project is to study mechanisms of immunity, immune evasion and immunopathology in schistosomiasis with the ultimate goals of developing an experimental vaccine suitable for human trials as well as understanding the pathogenesis of disease.

A. Role of interleukin-5 in schistosome induced eosinophilia and granuloma formation. Interleukin-5 (IL-5) was shown to be essential for parasite induced blood and tissue eosinophilia in mice. Nevertheless, infected mice depleted of this cytokine developed normal granulomas and fibrosis.

B. Effect of cytokine depletion on protective immunity. Protective immunity in mice was shown to be partially ablated by treatment with anti-IFN-γ antibodies whereas IL-5 depletion had no effect.

C. Cytokine responses of T cells in immunity and immunopathology. T cells from acutely infected mice or isolated granulomas were shown to produce cytokines (e.g. IL-5) characteristic of TH-2 lymphocytes whereas T cells from vaccinated mice produce predominantly TH-1 (e.g., IFN-γ) cytokines.

The above findings suggest that preferential induction of T cells belonging to the TH-1 subset may be a useful strategy for improving vaccine induced immunity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00253-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Studies of the Immunological Responses to Filarial Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D. Freedman	Medical Staff Fellow	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID
	N. Raghavan	Fogarty Fellow	LPD, NIAID
	E. Lobos	Fogarty Fellow	LPD, NIAID
	A. Limaye	Howard Hughes Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID

COOPERATING UNITS *(if any)* Indian Cncl of Med Res, Madras, India (S Tripathy, V Kumaraswami); Anna Univ, Madras, India (K Jayaraman); Thanjuvar Med Coll, Thanjuvar, India (S Jamal); Aga Khan Med Ctr, Karachi, Pakistan (R. Hussain); Dept of Hlth, Guatemala (G Zea-Flores); Mich St Univ (J. Williams); Univ S Fla (A Vincent); Uniformed Services Univ of the Health Sciences (R Lal).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	1.8	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with lymphatic filariasis, onchocerciasis, and loiasis.

IgG4 antibodies, an unusually prominent component of the response to chronic helminth infections, have close regulatory and functional linkages to IgE antibodies; indeed, current studies have identified them as the 'blocking antibodies' responsible for inhibiting allergic reactions to parasite antigens. Furthermore, since this subclass is 'restricted' from recognizing epitopes such as phosphocholine, a common constituent of parasitic and other infectious organisms, serologic assays for filarial infection based on IgG4 antibody detection have proven to be much more specific than those detecting the total IgG antibody response to filarial antigens.

Populations with bancroftian filariasis or onchocerciasis have been examined to define immunologic parameters that distinguish "naturally immune" from infected individuals. A 43kD protein from infective larvae appears as a potential protective immunogen for bancroftian filariasis, and antisera raised to this antigen and used to probe a W. bancrofti expression library have identified recombinant proteins whose value as protective immunogens now must be assessed. Similar studies in onchocerciasis have also identified such differentially recognized molecules, and antibodies are being raised to them.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00255-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Immunologic Responses to Non-Filarial Parasitic Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D.O. Freedman	Medical Staff Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID

COOPERATING UNITS *(if any)*

Aga Khan Medical School, Karachi, Pakistan (R. Hussain).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To account for the neo-arteriolization of the peri-portal fibrotic tissue around the eggs of Schistosoma mansoni in the livers of infected humans, parasite products were evaluated for endothelial cell growth factor activity. Very potent endothelial cell activation and proliferation were induced by soluble antigens from eggs (SEA) but not adult schistosomes, all in the absence of any immune cells or factors. Simple characterization studies indicated that the activity of SEA depends on a peptide moiety for at least part of its function, but the molecule itself has not been defined.

Serial serum specimens from patients with acute schistosomiasis mansoni have been studied for the evolution of their IgG and IgE antibody responses to different schistosome antigens. Because of the importance of IgG4 antibodies for specific diagnosis and for blocking allergic reactions to the parasite, this unique collection of sera is being used to define the kinetics of a developing specific IgG4 antibody response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00256-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Differentiation of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Others:	R. da Silva	Fogarty Fellow	LPD, NIAID
	S. Puentes	Clinical Staff Fellow	LCI, NIAID
	P. Pimenta	Fogarty Fellow	NCI

COOPERATING UNITS (if any)

Dr. Sam Turco, Dept. of Biochemistry, University of Kentucky

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	1.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

During growth within the sand fly and within axenic culture, Leishmania promastigotes undergo differentiation from a non-infective to an infective or metacyclic stage which is uniquely adapted for life in the vertebrate. For some species (e.g. L. major and L. donovani) this development is accompanied by loss of agglutination by the lectin peanut agglutinin (PNA). On non-infective, logarithmic promastigotes of L. major, PNA binds to the surface lipophosphoglycan (LPG) which is the major surface glycoconjugate of these cells. During metacyclogenesis, the LPG is developmentally modified such that it no longer binds PNA, it expresses a novel carbohydrate epitope, and it is substantially increased in size. Carbohydrate analyses of organically extracted metacyclic LPG revealed an approximate doubling in the number of the phosphorylated saccharide units, and a modification in the composition of the phosphorylated tetrasaccharides. Immunogold labeling of the surface LPG revealed a 10nm thickening in the LPG coat, which assumed a filamentous appearance on freeze-fracture preparations. These changes promote complement resistance by preventing insertion of the membrane attack complex into the membrane. The LPG, nonetheless, continues to activate complement efficiently, which is thought to promote intra-macrophage survival by facilitating attachment to and uptake by macrophages via complement receptors (CR1) which fail to trigger a respiratory burst. Opsonization of metacyclic promastigotes using anti-LPG monoclonal antibodies as opposed to complement opsonization resulted in intracellular killing, suggesting that the choice of receptors is critical to subsequent intracellular survival.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00257-08 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F.A. Neva	Chief	LPD, NIAID
Others:	P.J. Brindley	Special Volunteer	LPD, NIAID
	S. Greenberg	Medical Staff Fellow	MB, NCI
	W. Blattner	Chief, Viral Epid. Sect.	EEB, NCI
	L. Kramer	Special Volunteer	LPD, NIAID
	T. Nash	Senior Investigator	LPD, NIAID

Others continued below.

COOPERATING UNITS *(if any)*

Univ. of California at San Francisco, Dept. Pathology (J. McKerrow); SEMA, Inc., Rockville (G. Phillips); Univ. of the West Indies, Jamaica (Prof. Morgan, Ralph Robinson and S. Terry); Project SIDA, Kinshasa, Zaire (J. Perriens); Georgetown Univ. contract (W.T. London)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves both clinical studies of humans infected with the intestinal nematode, Strongyloides stercoralis, and basic analysis of parasite antigens and immunologic responses to them. The parasite has unusual biologic properties and can produce serious, even fatal, disease in certain immunosuppressive states.

A new supply of skin test antigens from infective larvae (L3) of S. stercoralis has been produced, and final analysis of immediate hypersensitivity skin tests obtained with the initial antigens is underway. The metalloprotease from L3 stage parasite larvae has been partially purified and further characterized. Polyclonal and monoclonal antibodies against the protease have been produced and it is hoped that affinity purification of the enzyme can be accomplished.

Clinical studies have been primarily focused upon gathering more information concerning the relationship of strongyloides infections in patients infected wit HTLV-1 virus. Infection with S. stercoralis appears to be common in these patients and difficult to eradicate. Preliminary information suggests that, in contrast, the parasite is not an important opportunistic infection in patients infected with the related retrovirus, HIV, which causes AIDS.

Other investigators cont.

E. Ottesen	Senior Investigator	LPD, NIAID
T. Fleischer	Chief, Clin. Immun. Service	CPD, Clin. Center
C. Brown	Medical Staff Fellow	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-07 LPD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. W. Cheever	Chief, Host-Parasite Relations	LPD, NIAID
Others:	J. Macedonia	Bio. Lab. Tech.	LPD, NIAID
	Y. Xu	Visiting Fellow	LPD, NIAID
	A. Sher	Head, Immunology	LPD, NIAID
	J. M. Grzych	Special Volunteer	LPD, NIAID
	P. Scott	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*

Laboratoire de Pathologie Cellulaire du Foie, Institut Pasteur, Lyon; France (Jean-Alexis Grimaud)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Treatment of mice with anti-IL-5 monoclonal antibody depleted S. mansoni-infected mice of eosinophils. Granulomas without eosinophils were only slightly reduced in size and had fibrosis equivalent to that in untreated mice. Treatment with anti-IFN-γ or anti-IL-4 also had no effect on granuloma size or hepatic fibrosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00350-07 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	R. Adam	Assistant Professor, Medicine	University of Arizona
	M. Mowatt	Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analysis and comparison of partial cDNA sequences of Giardia genes encoding two variant major surface antigens of a clone of WB and one of its progeny revealed both had high cysteine content and cys-x-x-cys motifs. The confirms other studies showing preferential incorporation of cysteine into the variant surface antigens. No other homology has been found thus far. Two other genes were identified and sequenced. A structural gene encoding for one of the "giardin" proteins and a heat shock protein of 1470 nucleotides. The giardin gene is found in all Giardia isolates studied while the heat shock gene is not present in a subset of Giardia previously noted to be unique by other criteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00351-07 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	P.A. Scott	Expert	LPD, NIAID
Others:	A. Sher	Section Head	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID
	P. Natovitz	Biologist	LPD, NIAID
	D. Dwyer	Supvr. Microbiologist	LPD, NIAID
	E. Pearce	Visiting Associate	LPD, NIAID
	S. Heath	Guest Researcher	LPD, NIAID
	M. Knight	Guest Researcher	LPD, NIAID

COOPERATING UNITS (if any)

DNAX Research Institute, Palo Alto, CA (R. Coffman).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The objectives of this project are to develop a vaccine against cutaneous leishmaniasis by both defining protective leishmanial antigens and investigating the mechanisms responsible for protective immunity using experimental infections of BALB/c mice with Leishmania major. One antigen fraction (9) induces protection, is recognized by a T cell line that adoptively transfers protection and belongs to the TH1 subset of CD4+ cells. In contrast, another antigen fraction (1) was not protective and stimulated the TH2 CD4+ subset. We have established additional T cell lines that recognize these two antigen fractions, and have confirmed the strong association of fraction 9 with protection and TH1 T cells, and fraction 1 with exacerbation and TH2 T cells. In addition, we analyzed the lymphokines produced by T cells from infected mice that had been immunized with the different fractions. T cells from animals protectively immunized with fraction 9 produce large amounts of IFN-gamma, while T cells from fraction 1 immunized mice produce elevated levels of IL-4. Taken together, these results suggest that certain parasite antigens induce protection by stimulation of TH1 cells, while other parasite antigens counteract protective T cells by stimulation of the TH2 subset of CD4+ T cells. Furthermore, we found that the mechanism by which TH2 cells exacerbate infection may be by modulation of IFN-gamma induced activation. Thus, macrophages exposed to IL-4, GM-CSF, and/or IL-3 exhibit decreased parasite killing when activated with IFN-gamma. Three T cell clones, all of the TH1 phenotype, have been established to identify protective antigens. One clone, 9.1-2, recognizes a 10,000 m.w. protein and is protective. In addition to recognizing a L. major antigen, this clone recognizes antigens from L. mexicana, L. braziliensis, L. panamensis, and L. donovani, but not Trypanosoma cruzi or Crithidia fasiculata. Finally, we have found that the protein recognized by this clone is released by promastigotes during in vitro culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00439-05 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D.O. Freedman	Medical Staff Fellow	LPD, NIAID
	C. King	Medical Staff Fellow	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)* Indian Council of Medical Research, Madras, India; (R. Prabhakar and V. Kumaraswami); Madras Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (M. Mulligan); Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil (A. Coutinho, G. Dreyer); Dept of Health, Cotonou, Benin (J.P. Manshande).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Ivermectin has been shown in trials in South India to be effective in clearing microfilaremia in patients with bancroftian filariasis at dosages as low as 20-mcg/kg given once orally. A second placebo-controlled trial of ivermectin and the currently used drug, diethylcarbamazine (DEC), has indicated that the efficacy of ivermectin and DEC was equivalent for 3 months, but by 6 months the ~30% microfilarial recurrence of the ivermectin-treated patients was significantly greater than the ~15% recurrence of the DEC group. Side effects were qualitatively and quantitatively identical for both groups. Thus, though ivermectin appeared somewhat less effective than DEC at 6 months, its single-oral-dose mode of administration with toxicity no greater than that of DEC means that this drug should engender greater patient compliance and, therefore, be much more effective in mass-treatment filariasis control programs than DEC. Current trials are underway to determine the lowest effective dose of drug in both South India and Brazil.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00483-04 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Drug Resistance and Red Cell Invasion in Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas E. Wellems	Sr. Staff Fellow	LPD, NIAID
Others:	David Peterson	Research Fellow	LPD, NIAID
	Stephen Dolan	Med. Staff Fellow	LPD, NIAID
	Annie Walker-Jonah	Fogarty Fellow	LPD, NIAID
	Lindsey J. Panton	Fogarty Fellow	LPD, NIAID
	Cecilia Graziosi	Special Volunteer	LPD, NIAID

COOPERATING UNITS (if any)

Washington University, St. Louis, MO (Donald J. Krogstad, Ilya Gluzman); Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. (Wilbur K. Milhous)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our investigations are directed at understanding the mechanisms of drug resistance and red blood cell invasion by the malaria parasite Plasmodium falciparum. Crosses of cloned parasite lines and linkage analysis are being employed to identify these mechanisms. Such an approach has shown that point mutations at the active site of dihydrofolate reductase confer resistance to two common antimalarials, pyrimethamine and proguanil. Different mutations affect the response to each of these drugs independently, explaining at a structural level why strains refractory to one drug may still respond to the other.

Chloroquine resistance is being examined through a cross of two geographically distant P. falciparum strains (from Central America and Indochina). Independent recombinant clones which have been isolated from this cross show an even distribution of chloroquine resistant and sensitive forms. All resistant progeny have the same phenotype as the resistant parent. Linkage data show no correlation between inheritance of chloroquine resistance and a P. falciparum P-glycoprotein ("mdr") gene. The data instead suggest that a single genetic locus on another chromosome confers resistance to the progeny.

A molecular-genetic approach is also being used to explore factors affecting red blood cell invasion. Neuraminidase treatment of target red cells has shown that the parents of one of our crosses have a large difference in their dependence on sialic acid residues for invasion. Examination of the progeny should now reveal fundamental information about the genetics of invasion pathways involving glycophorin. We have also found in two crosses that progeny which inherit a more rapid proliferation rate from one parent are favored in infection. RFLP analysis links this rapid proliferation to a factor (possibly a gene encoding a histidine-rich protein) near a telomere of chromosome 13.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00487-03 LPD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Quantitative Parasitology of Schistosome Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. W. Cheever	Chief, Host-Parasite Relations	LPD, NIAID
Others:	J. Macedonia	Bio. Lab. Tech.	LPD, NIAID

COOPERATING UNITS *(if any)*

Laboratory of Statistical Methodology, DCRT (J. Mosimann)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Present efforts are concentrated on evaluating the role of host reactions on parasitologic parameters (see Z01-AI-00347) and on the development of models for the kinetics of egg laying, egg destruction, and egg excretion in S. mansoni and S. japonicum infections. Accurate assessment of the rate of egg destruction is important for these models. Eggs in the tissues of S. japonicum-infected mice persisted in unchanged numbers in the tissues for at least 1 year after chemotherapeutic cure of infections of 8 weeks' duration. The destruction of S. mansoni eggs in murine tissues may also be slower than previously thought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00494-03 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of T Cell Responses in Human Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Others:	P. Melby	Staff Fellow	LPD, NIAID
	F. Neva	Chief	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunity to infection by the intracellular parasite Leishmania is mediated by sensitized T cells, however direct recognition of infected macrophages by T cells has not been clearly demonstrated. Experiments addressing this question have been confounded by the possibility that T cell activation in these systems might be due to release of leishmanial antigens by infected cells and subsequently presented by contaminating populations of uninfected antigen presenting cells (APCs) such as B cells or uninfected macrophages. We have addressed this issue using peripheral blood mononuclear cells (PBMNCs) from patients with healing cutaneous L. major infection. Monocytes were purified from PBMNCs and then infected with serum opsonized L. major or L. donovani amastigotes. Cells were then lightly fixed to stop active infection and prevent further processing and possible release of parasite antigens. Addition of functionally pure autologous T cells resulted in strong proliferative responses to both L. major and L. donovani fixed infected monocytes. This stimulation could not be augmented by the addition of uninfected monocytes. Supernatents from infected monocytes failed to stimulate unfractionated PBMNCs indicating the absence of non-cell associated soluble antigens. We conclude that Leishmania infected macrophages are able to efficiently process and present antigens to sensitized T cells.

Identification of the antigens which are being recognized by T cells from immune individuals is continuing using previously generated T cell clones. Leishmanial antigens separated by 2-D electrophoresis as well as FPLC and phenyl-sepharose chromatography have been used to identify specific proteins which activate a number of these clones.

The development of DNA probes for cytokines produced by activated lymphocytes and macrophages has been completed and they will now be used to reexamine the responses of lymphocytes from patients with cutaneous, mucocutaneous or visceral disease to defined leishmanial antigens or to autologous infected macrophages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00512-02 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Definition of Filarial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. B. Nutman	Senior Investigator	LPD, NIAID
Others:	N. Raghavan	Visiting Scientist	LPD, NIAID
	E. Lobos	Visiting Scientist	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID
	E. A. Ottesen	Senior Investigator	LPD, NIAID

COOPERATING UNITS *(if any)*

New England Biolabs, Beverly, Mass (F. Perler, L. McReynolds, C. Maina)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.7	2.7	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the study of the human filariases, progress has been hampered by 1) the lack of defined parasite antigens; 2) the broad immunological cross-reactivity seen among the eight filarial species of humans; and 3) the dearth of abundant parasite material. The objectives of this project are to define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species.

cDNA and genomic libraries have been either constructed (Brugia malayi, Loa loa and Wuchereria bancrofti, Onchocerca volvulus) or made available (Onchocerca volvulus, Brugia malayi larval cDNA library) so that screening with defined patient sera, patient T cells or parasite DNA could be performed. Recombinant antigens and probes have been identified that: a) induce T cell responses in an antigen-specific manner; b) may be in part protective in onchocerciasis; c) can distinguish among related filarial species by restriction fragment length polymorphisms, PCR or direct Southern blotting; d) encode immunoreactive and potentially protective molecules of W. bancrofti; e) identify repeated segments of the either the W. bancrofti genome or that of Loa loa; and f) may be of potential diagnostic importance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00517-02 LPD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ligand-receptor Interactions in Parasite Attachment to Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	Steve Fuhrman	Medical Staff Fellow	LPD, NIAID
	G. Furtado	Guest Researcher	LPD, NIAID
	J. Schweinle	IPA	LPD, NIAID
	G. Ogden	Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

A. Tenner, American Red Cross; A. Ezekowitz, Children's Hospital, Harvard Univ.; H. Kleinman, LSBA, NIDR; Y. Yamada, LSBA, NIDR; H. Krivan, LSB, NIADDK

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.1	3.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major ligand-receptor systems involved in uptake of the intracellular pathogens Trypanosoma cruzi, Salmonella, and Toxoplasma gondii by phagocytic and non-phagocytic cells were examined.

Earlier experiments with T. cruzi were extended by showing that purified C1q enhances uptake of trypomastigotes but not epimastigotes into human foreskin fibroblasts. The acute phase reactant, mannose binding protein (MBP), binds to mannose rich but not mannose poor isolates of Salmonella. MBP bound to Salmonella functions as a direct opsonin for ingestion by neutrophils, possibly by binding to neutral glycolipids in the neutrophil. MBP, at concentrations found in normal human serum, augments deposition of C3 on the Salmonella by over 3 fold. Despite the overall structural similarity between MBP and classical complement pathway subcomponent C1q, augmentation is mediated entirely by the alternative pathway. MBP sensitization converts organisms ordinarily resistant to serum bactericidal activity to serum susceptible status.

The basement membrane protein, laminin, markedly augments attachment and entry of tachyzoites of Toxoplasma gondii into macrophages and fibroblasts. Laminin binds to a 65-60kD surface iodinatable protein on tachyzoites which is not extractable with non-denaturing detergents. This molecule is recognized by an antibody to the ubiquitous 67kD laminin binding protein present on a variety of mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00535-02 LPD

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Characterization of Intracellular Compartments Containing Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	B.F. Hall	Medical Staff Fellow	LPD, NIAID
	J.E. Schweinle	IPA	LPD, NIAID
	D. Rotrosen	Medical Staff Fellow	LCI, NIAID
	A.B. Tartanian	Technician	LPD, NIAID
	Steve Fuhrman	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*
Ira Mellman, Associate Professor, Yale Univ.; Lloyd Kasper, Associate Professor, Dartmouth College; John Albert, Technician, Georgetown Univ.; Tomas Ganz, Associate Professor, UCLA

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.8	1.7	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Our laboratory is interested in purifying and characterizing intracellular compartments containing pathogenic microorganisms.

Experiments were completed on the immunochemical characterization of neutrophil phagosomes containing Salmonella typhimurium. Our results show that cell surface phagocytic receptors and azurophil granule components are indiscriminately incorporated into phagosomes regardless of the ligand on S. typhimurium. In contrast, specific granule constituents are 4-5 fold more prominent in phagosomes containing IgG coated Salmonella than in phagosomes containing C3 coated Salmonella.

Experiments were completed on the characterization of phagosomes in J774 cells containing Trypanosoma cruzi. Using both intracellular iodination and immunofluorescence, we showed that non-infective epimastigotes are internalized into vacuoles which contain the complement receptor CR3, whereas infective metacyclic trypomastigotes reside in CR3 negative vacuoles. Both compartments contain lysosome-specific glycoproteins, indicating that the parasitophorous vacuole has fused with lysosomes.

Experiments were completed on characterization of parasitophorous vacuoles in Chinese Hamster Ovary (CHO) Cells containing Toxoplasma gondii. Native parasites entering CHO cells reside in compartments which neither acidify nor fuse with lysosomes or endosomes. Antibody coated or heat killed tachyzoites entering CHO cells transfected with the murine FcRII receptor reside in compartments which acidify and are fusigenic.

These results illustrate three different forms of selectivity in incorporation or exclusion of membrane receptors and/or granule constituents into phagosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00536-02 LPD

PERIOD COVERED

October 1, 1988- September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Transcriptional Regulation in Trypanosoma cruzi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Keith Joiner	Senior Investigator	LPD, NIAID
Others:	G.B. Ogden	Staff Fellow	LPD, NIAID
	B.F. Hall	Medical Staff Fellow	LPD, NIAID
	G. C. Furtado	Guest Researcher	LPD, NIAID

COOPERATING UNITS *(if any)*

none

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Unit of Microbial Pathogenesis

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in understanding the molecular mechanisms controlling gene expression in Trypanosoma cruzi. We have attempted to identify, in T. cruzi, genes which might share DNA homology with known transcriptional activators in yeast (e.g., GCN4) and mammalian cells (e.g., c-fos and c-myc). We found that T. cruzi DNA, in dot-blot hybridization experiments, hybridizes with the c-fos- and GCN4-specific probes, but not with a probe specific for c-myc. Further analysis by Southern hybridization revealed that the c-fos probe hybridizes to a single 8.1 kb band in EcoRI-digested T. cruzi DNA. The cloning and sequencing of this 8.1 kb DNA fragment should allow the identification of any c-fos-related genes contained within it. Moreover, we would like to study the regulation of this putative fos-related gene in vivo. Towards this end, we have initiated experiments with the ultimate goal of developing a plasmid-based transformation system in T. cruzi. We have been able to grow the parasite on a solid medium, which allows us to detect foreign DNA in the parasite by colony hybridization experiments. We have successfully transformed T. cruzi with plasmid DNA, and reisolated this DNA from parasite nuclei. Finally, we have determined that T. cruzi protein synthesis and parasite multiplication ceases in the presence of the antibiotic Geneticin. Parasites resistant to the drug have not been apparent. Thus, the gene (neo) conferring resistance to this drug may be useful as a resistance marker in a T. cruzi-based plasmid vector.

LVD

LABORATORY OF VIRAL DISEASES
1989 ANNUAL REPORTS
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LABORATORY OF VIRAL DISEASES
Annual Reports, 1989
SUMMARY

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. These studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of virus infections. Current topics of basic research include regulation of gene expression, mechanisms of DNA replication, assembly and transport of virus particles, action of virus growth factors, determinants of virus virulence, host resistance genes, and viral targets of humoral and cellular immunity. More applied areas of investigation include the development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of viruses including human immunodeficiency virus.

REGULATION OF VIRUS GENE EXPRESSION

DNA viruses provide useful systems for studying basic aspects of gene expression and can be used as expression vectors. Several different virus systems are currently under investigation by members of LVD.

Poxviruses. Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. These features make poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. Additional information regarding transcription also is needed to improve vaccinia virus expression vectors, which are currently being tested as candidate live vaccines against several diseases including AIDS. During the past year, progress has been made in determining the signals used for initiation of transcription of early and late vaccinia virus genes and the enzymes and factors needed for expression.

The structure of vaccinia virus early and late promoters. Synthetic oligonucleotides were used to examine the effects of mutations on early and late promoters. From an analysis of the expression of over 450 recombinant viruses, a relatively simple picture emerged. Early promoters consist of 3 regions: a 15 base pair critical region in which most single nucleotide substitutions have a major effect on expression, separated by 11 base pairs of a less critical T-rich sequence from a 5 to 7 base pair sequence within which initiation with a purine usually occurs. Late promoters consist of a T- or A-rich sequence of about 20 base pairs, a spacer region of about 6 base pairs, and a highly conserved critical TAAAT sequence containing the RNA start site, which is usually followed by G or A nucleotides.

(Davison, Moss)

Early and late transcription factors. Transcription of early genes can be carried out *in vitro* using RNA polymerase and a 150,000 Dalton transcription factor, both of which have been purified from infectious virus particles. Transcription of late genes has been accomplished only with extracts from virus-infected cells. The infected cell extract has been separated by phosphocellulose chromatography into 3 fractions required for transcription. One late transcription factor with an apparent molecular weight of 45,000 has been partially purified.
(Wright, Moss)

Identification of the vaccinia virus genes encoding capping enzyme. The vaccinia virus capping enzyme is composed of subunits of approximately 95,000 and 31,000 Daltons. The large subunit was previously mapped to the D1 open-reading-frame on the vaccinia virus genome. Immunological procedures have now been used to identify D11 as the gene encoding the small subunit.
(Broyles, Shuman, Moss)

The vaccinia virus gene encoding topoisomerase is essential for virus replication. Vaccinia virus encodes a type 1 topoisomerase. Nothing is known regarding the role of this enzyme in the virus life cycle. To determine whether the topoisomerase is essential, we attempted to interrupt the gene by inserting a selectable marker. Analysis of the genome structure of drug-resistant viruses revealed that, in each case, both the wild-type and mutant alleles were present. Retention of the wild-type allele is evidence that topoisomerase is essential.
(Shuman, Moss)

Parvoviruses. Parvoviruses are small viruses that contain a single-stranded DNA genome. One well studied member of this family, adeno-associated virus (AAV), is dependent for replication on coinfection with an adenovirus or herpesvirus. AAV gene expression appears to depend on both cellular and helper virus products.

Regulation of expression of AAV structural and non-structural proteins. Previous data suggesting that structural protein B is initiated from an ACG initiation codon has been confirmed by site directed mutagenesis. Site directed mutagenesis also was used to confirm the derivation of structural protein A from an alternately spliced transcript. All 3 structural proteins are required for infectivity, since mutants that fail to produce any one of the three are not infectious. Some of the non-structural proteins are derived from unspliced RNAs, whereas others are derived from spliced products.
(Sebring, Wong, Muralidahar, Rose)

VIRUS DNA REPLICATION

Viruses provide useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus encoded factors provide potential targets for chemotherapy.

Poxviruses. Poxviruses provide a unique experimental system, since the enzymes and factors are encoded within the virus genome and replication occurs in the cytoplasm. Previous studies revealed that vaccinia virus DNA is replicated as concatemeric structures.

Discontinuous sequence required for resolution of the concatemer junction of vaccinia virus replicative DNA. The concatmer junction from vaccinia virus replicative intermediates was cloned into bacterial plasmids and, after transfection into cells infected with vaccinia virus, was replicated and resolved into linear minichromosomes with sealed terminal hairpins. The introduction of a series of symmetrical insertions, deletions, and site directed oligonucleotide substitutions has demonstrated that a sequence consisting of ATTTA and CTAGAAAAAAA separated by 4 or 5 nucleotides, located on both sides of the apex segment, is critical for resolution.

(Merchlinsky)

Drug-resistance domains of vaccinia virus DNA polymerase. Previous work established that substitution of methionine for leucine at residue 645 of DNA polymerase confers resistance to the drug aphidicolin. To further analyze the drug-resistance domain, attempts were made to isolate additional aphidicolin-resistance mutants by substituting other amino acids for leucine. No additional mutants were obtained.

(DeFilippes)

Herpes simplex virus. Herpes simplex virus (HSV) and other members of the herpesvirus family are significant human pathogens. The study of HSV DNA synthesis is useful as a model for eukaryotic DNA replication and for designing new anti-viral strategies. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication. Other studies have concentrated on the DNA signals required for DNA packaging.

Biochemical characterization of the HSV DNA binding protein (UL9). DNase footprint analysis has shown that the UL9 protein interacts with two related sites located on each arm of a nearly perfect palindrome within the HSV-1 origin of DNA replication. Evidence for a cooperative interaction between two bound UL9 molecules was obtained. In addition to origin binding, the purified UL9 protein possesses an intrinsic 3′ to 5′ helicase activity which might function in a critical early step in initiation of replication.

(Olivo, Challberg)

Characterization of the HSV-1 DNA polymerase holoenzyme. The DNA polymerase purified from HSV-1 infected HeLa cells was shown to consist of a complex of at least two polypeptides: UL30, the catalytic subunit, and UL42. Although the UL30 protein alone has catalytic activity using nicked DNA as a template, the complex is much more active on a single-stranded DNA template primed with a short oligonucleotide.

(Gottlieb, Challberg)

Identification of an HSV-encoded helicase-primase. In collaboration with workers at Stanford, a helicase-primase containing 1:1:1 products of three genes -- UL52, UL5, and UL8 -- was identified.
(Olivo, Sherman, Challberg)

Signals required for cleavage and packaging of HSV genomes. Cleavage of viral concatemers is coupled to their packaging into nucleocapsids. Two *cis*-acting signals are required. Present studies indicate that there is a required polarity for the two signals with cleavage occuring at a defined distance from them.
(Lum, Frenkel)

Adeno-associated virus

Replication of AAV depends of helper adenoviruses or herpesviruses.

Role of HSV genes in enhancement of AAV DNA replication. AAV DNA synthesis, measured using an infectious AAV2 plasmid, could be induced maximally with any six of the seven HSV genes required for HSV replication plus the immediate early HSV gene ICP4. No one of the seven HSV-required genes seemed absolutely essential, however. (Rose, Challberg)

SYNTHESIS, ASSEMBLY, AND TRANSPORT OF VIRAL PROTEINS AND PARTICLES

Information regarding the folding, asssembly, and transport of viral glycoproteins and the formation of virus particles is of general interest and practical importance, since it could lead to a greater understanding of antigenic structure. This information is critical for designing vaccines that induce neutralizing antibody.

Influenza and vesicular stomatitis viruses. The influenza hemagglutinin (HA) and the vesicular stomatitis virus (VSV) G protein are the best characterized viral envelope proteins and, therefore, serve as excellent models. Additionally, influenza virus remains a significant human pathogen.

Brefeldin A (BFA) reversibly induces the redistribution of both resident and itinerant *cis-* and medial-Golgi complex proteins to the endoplasmic reticulum (ER). BFA is a fungal metabolite that blocks the transport of newly synthesized proteins to the plasma membrane. Thus, in the presence of drug, both HA and G proteins are localized to the ER of infected cells. Surprisingly, despite the ER localization of these proteins, their N-linked ologosaccharides are modified by enzymes associated with the *cis-* and medial Golgi complex, but not by *trans* Golgi complex-associated enzymes. These paradoxical findings are best explained by the redistribution of proteins from the *cis-* and medial Golgi complex to the ER.
(Russ, Doms, Yewdell)

Human immunodeficiency virus (HIV). HIV contains an envelope glycoprotein composed of two subunits -- gp120 and gp41 -- that is formed by processing of a gp160 precursor. The envelope protein facilitates the entry of HIV by binding to the CD4 receptor on lymphocytes and is a target of neutralizing antibody.

Oligomeric structure of the HIV envelope glycoprotein. Pulse-chase studies indicated that gp160 is synthesized as a monomer and then assembles post-translationally into an oligomeric form. The sedimentation value of the oligomer is consistent with its being a homodimer. Mutagenesis studies suggest that the region most important for dimer stability resides in the ecto- and/or transmembrane domain of gp41. There is additional evidence for larger but less stable oligomeric forms. Parallel experiments also have been carried out with HIV-2 and SIV envelope proteins.

(Earl, Doms, Chakrabarti, Moss)

Determination of the HIV gp120-binding region of the CD4 molecule. CD4 is a surface molecule of human helper T lymphocytes that serves as the receptor for HIV. To map the binding site, mutant forms of CD4 containing a 2 amino acid insertion at different locations were synthesized. Impaired gp120 binding resulted from insertions within the first domain, particularly in the region corresponding to the CDR2 loop of the immunoblobulin light chain.

(Mizukami, Berger, Moss)

HIV-like particles produced by a vaccinia virus expression vector. Immature and mature retrovirus-like particles were shown to bud from cells that were infected with a recombinant vaccinia virus that expresses the entire *gag-pol* precursor protein from HIV-1. The mature particles contained reverse transcriptase as well as processed p24 and p17 *gag* polypeptides. Thus, assembly and maturation of HIV-like particles can occur in the absence of either infectious RNA or envelope protein. Such non-infectious particles could provide a safe source of material for the development of vaccines.

(Karacostas, Moss)

VIRUS-HOST INTERACTIONS

Molecular virology studies have largely focused on the virus itself. The role of virus/host interactions in determining pathogenesis and host immunity is now receiving greater emphasis in LVD.

Vaccinia virus encodes a secretory polypeptide structurally related to complement control proteins. A polypeptide with a molecular weight of approximately 35,000 is secreted from vaccinia virus-infected cells. The gene encoding this protein was found to belong to the 60-amino acid repeat superfamily and to have marked sequence similarity to the human complement 4b binding protein. *In vitro* studies indicate that the vaccinia protein inhibits the classical complement pathway. It seems likely that the role of this protein is to protect the virus against the host immune system.

(Kotwal, Moss)

Evidence for a cowpox virus gene that modulates the host inflammatory response. Mutation of a cowpox virus gene encoding a 38,000 molecular weight protein has a profound effect on the pocks formed on the chick embryo chorioallantoic membrane Macroscopically, the

mutant pocks are white instead of red. Microscopically, the white pocks are infiltrated with large numbers of heterophils and macrophages, which correlated with a reduction in the level of virus antigen and infectivity. By contrast, the red pocks were characterized by a relative absence of inflammatory cells and increased levels of virus.
(Palumbo, Buller)

VIRAL IMMUNOLOGY

Cytotoxic T cells (CTLs) play an important role in eradicating intracellular pathogens and cancer cells. It is well established that processed antigens must associate with MHC class I molecules, apparently by binding to a groove within the latter.

Viral determinants associate with class I MHC molecules within an intracellular compartment, most likely the endoplasmic reticulum (ER). This assertion is based on three lines of evidence. First, the effect of protein synthesis inhibitors on the presentation of cytosolic viral protein antigens can be correlated with the half-time of transport of MHC molecules from the ER. Second, presentation of viral protein antigens is completely blocked by brefeldin A. Third, presentation of vaccinia virus antigens is inhibited by the co-expression of the adenovirus E19 protein that binds class I MHC molecules in the ER.
(Cox, Eisenlohr, Yewdell, Bennink)

Localization of human CTL epitopes on HIV-1 reverse transcriptase. A series of recombinant vaccinia viruses that express truncated HIV-1 reverse transcriptase genes were constructed and used in collaborative studies to locate the epitopes recognized by human CTL clones. Further epitope mapping was achieved using a series of overlapping 25 amino acid synthetic peptides. In this manner, four different epitopes were identified, each resticted by a different HLA class I antigen.
(Flexner, Moss)

VIRUS EXPRESSION VECTORS

The use of expression vectors has become an important part of recombinant DNA technology. Vaccinia virus is now widely used for expression of proteins in mammalian cells and has proven particularly useful for determining the targets of humoral and cellular immunity. Recombinant vaccinia virus is currently being tested as a candidate AIDS vaccines in humans and as a rabies vaccine in wild-life. The unique DNA integration properties of some parvoviruses suggest that they would have valuable uses as vectors.

Improvements in the hybrid vaccinia virus/bacteriophage T7 expression sytem. A hybrid expression system employing a recombinant vaccinia virus that expressses bacteriophage T7 RNA polymerase was developed. Differences in the structure of the 5'ends of prokaryotic and eukaryotic mRNAs, however, have limited the efficiency of the system. By employing the untranslated leader sequence of encephalomyocarditis virus, the translation of mRNA made by bacteriophage T7 RNA polymerase was greatly increased. This modification has made the vaccinia/T7 vector one of the highest expressing eukaryotic systems.

(Elroy-Stein, Moss)

Transfer of the inducible *lac* repressor/operator system from *E. coli* to a vaccinia virus expression vector. Regulation of gene expression was obtained by attaching the *lac* operator to a vaccinia promoter and expressing the *lac* repressor gene. Expression was then achieved by adding the inducer IPTG. This modification should make it possible to express potentially toxic genes in vaccinia virus expression vectors.

(Fuerst, Moss)

Development of an adeno-associated virus (AAV) vector. A series of AAV-based vectors have been created in which the endogenous AAV promoters and coding sequences have been replaced with a polylinker and strong constitutive heterologous promoters. In addition, an encapsidation system has been developed to produce high titers of infectious virus.

(Chatterjee, Wong, Rose)

CHARACTERIZATION OF NEWLY DISCOVERED VIRUSES

Human herpesvirus 6. HHV-6 is a newly identified T lymphotropic herpesvirus that has been isolated from patients with AIDS as well as from healthy individuals. The virus appears to be the causative agent of exanthem subitum (roseola) in young children, but it is not known whether HHV-6 causes other diseases.

Molecular biology of human herpesvirus 6. Using methods adapted to the propagation, assay, and analysis of HHV-6, considerable variation has been found in the structure and biological properties of different isolates. A new virus strain (RK) was isolated following activation of latent virus from $CD4^+$ cells. IL-2 exhibits an interesting dose-dependent inhibitory effect on HHV-6 replication. The maturation of HHV-6 occurs in both the nucleus and cytoplasm, whereas the prototype member of the herpesvirus family, HSV, matures in the nucleus.

(Avidor, Danovich, Katsafanos, Roffman, Schirmer, Wyatt, Frenkel)

DEVELOPMENT OF ANTI-VIRAL AGENTS

Specific anti-HIV properties of CD4-*Pseudomonas* exotoxin hybrid protein. A recombinant molecule, containing the first two domains of CD4 linked to active active regions of *Pseudomonas* exotoxin A, has been expressed. The hybrid toxin binds to HIV-infected cells that express gp120 on their surface and selectively kills them. In addition, the toxin inhibits the spread of HIV to uninfected cells. In contrast, the toxin produced no direct cytotoxic effects or functional impairment of uninfected cells bearing MHC class II molecules.

(Berger, Mizukami, Moss)

ADMINISTRATIVE CHANGES

During the past year, those members of the Genetic Engineering and Macromolecular Biology Sections that were in Building 5 moved to Building 4, and members of the Molecular Structure Section moved from Building 5 to Twinbrook II. Drs. Mark Challberg and Niza Frenkel were approved for tenure by the Board of Scientific Directors and are now "permanent" members of LVD. As usual, there has been a healthy turnover of young investigators who came to NIAID to further their research training. Some have gone on to academic appointments and others to positions in biotechnology.

Honors, awards, and service. Members of LVD presented numerous invited lectures and seminars in this country and abroad. Dr. Moss received the Dickson Prize for Medical Research awarded by the University of Pittsburgh School of Medicine and gave the annual Mager Lecture at the Hebrew University-Hadassah Medical Center in Jerusalem. Drs. Rose and Moss are both on the editorial board of *Virology*, and Dr. Moss also serves on the editorial boards of *The Journal of Virology, AIDS Research and Human Retroviruses, Advances in Virus Research*, and *Current Opinion in Biotechnology*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00123-23 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Replication of Poxvirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Merchlinsky	Staff Fellow	LVD, NIAID
Others:	B. Moss	Chief	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Poxviruses provide a unique experimental system for studying DNA replication. Required enzymes and factors are encoded within the viral genome and DNA synthesis, and processing occurs within the cytoplasmic compartment of the cell. Analysis of a large series of temperature-sensitive mutants of vaccinia virus has allowed us to divide genome formation into two stages: DNA replication which generates concatemers, and resolution of concatemers into monomers. Expression of early genes is required for the former and late genes for the latter. Site-directed mutagenesis was used to define the sequence required for the resolution of the vaccinia virus concatemer junction. A model for resolution involving site-specific recombination and oriented branch migration is consistent with our data. The requirements for resolution and intramolecular recombination are different. Biochemical and genetic studies indicated that early functions, including DNA replication, are necessary for intramolecular recombination in transfected plasmids, but that late functions are not necessary. A DNA$^+$ temperature-sensitive mutant blocked in recombination was found.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00126-16 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Functional Analysis of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: F. DeFilippes Research Physicist LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

A vaccinia virus mutant that is resistant to the drug aphidicolin (AP) and that codes for an AP-resistant DNA polymerase has been described. A single base change at nucleotide position 2430 of the DNA polymerase gene changes leucine to methionine at amino acid residue 645 in the polymerase and confers AP resistance. The effects of nucleotide alterations at the site of the original mutation in the vaccinia virus genome have been studied. The results are that nucleotide alterations which code for amino acids other than methionine at residue 645 do not confer AP resistance to the virus. The methods which I developed for the rapid mutagenesis of this site in the vaccinia genome were based on the procedure developed by Eckstein; however, I believe that the polymerase chain reaction (PCR) is a more efficient method for site-directed mutagenesis than the Eckstein procedure. I have used the PCR to produce a double-stranded DNA product which confers the AP mutation, and I am currently producing PCR products which will alter the amino acids in the conserved region surrounding residue 645 in the polymerase. The PCR products with the above alterations also contain the AP resistance mutation so that AP will be used to select for the insertion of DNA with a change in the conserved region. The PCR will also be used to produce DNA for altering other regions of the polymerase gene to test for AP resistance and to create inserts for cloning into expression vectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00294-08 LVD

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS *(if any)*
R. McPherson, M.D., Department of Pathology, Georgetown University Hospital, Washington, DC; D. Klessing, Waksman Institute, Piscataway, NJ

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and function of certain sequences (e.g., the inverted terminal repeats) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specific functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Our results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electrophoresis, base sequence analysis, and DNA transfection.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI-00295-08-LVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.A. Rose Section HeadLVD, NIAID

COOPERATING UNITS *(if any)*

M. Challberg, Section Head LVD, NIAID; L. Mishra, Senior Staff Fellow, Division of Blood and Blood Products, CBER, FDA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.25	PROFESSIONAL:	OTHER: 1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are (i) to define where and how each required helper virus factor regulates expression of defective human parvovirus (AAV) genomes, and (ii) to relate these findings to their respective roles in the replication of the helper viruses (adenoviruses, herpesvirus) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses, and DNA transfection of both simian and human cell lines.

PHS 6040 (Rev. 1/84) GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00296-08-LVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID
	K. Wong	Medical Staff Fellow	LVD, NIAID
	S. Muralidahar	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory Of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.6	2.1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of these studies are (1) to identify and characterize all proteins that are specified by the defective human parvovirus (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (2) to define the mechanism(s) by which the AAV proteins arise, and (3) to define specific functions of the AAV proteins. We have identified several AAV non-structural proteins which were previously undetected. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins although they share large segments of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. Mechanisms that regulate expression of AAV proteins are of fundamental interest, and we have now shown that one AAV structural protein is initiated by a codon not known previously to act as an initiation codon in higher eukaryotes. Furthermore, our current findings (i) support a "scanning mechanism" in he translational expression of polycistronic eukaryotic mRNAs, and (ii) demonstrate that alternative mRNA splicing is required for effective translational expression of the largest AAV capsid protein. Among methods used are site-directed mutagenesis, affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, DNA transfection, immunoprecipitation, and Western blotting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZOI AI-00297-08-LVD

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID
	S. Chatterjee	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both in vivo and in vitro systems. We have shown that AAV DNA synthesis can be initiated in vitro in cell-free extracts and that replicating forms that correspond to those identified in vivo can be synthesized with either endogenous or exogenously added templates. Recently, we have identified two DNA-associated proteins that may play an important role in the required processing of self-primed unit length hairpin and concatemeric intermediates of DNA replication. The observed mode of AAV DNA replication may serve as model for telomere replication in eukaryotes. Among methods used are affinity chromatography, gel-electrophoresis, DNA sequence analysis, and restriction cleavage of DNA molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00298-08 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Chief	LVD, NIAID
Others:	O. Elroy-Stein	Special Volunteer	LVD, NIAID
	A. Hugin	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)* Molecular Vaccines

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vaccinia virus has been developed into a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. Several different methods have been developed to select or screen the recombinant vaccinia viruses. The recombinant viruses can then be used to express proteins in a variety of tissue culture cells or animals. During the past year, the inducible *lac* repressor/operator system from *Escherichia coli* was transferred to vaccinia virus expression vectors. It is now possible to selectively induce gene expression by adding isopropyl β-D-thiogalactoside. Inducibility now makes it possible to express toxic genes. A novel modification of the recombinant vaccinia virus expression system which utilizes the bacteriophage T7 RNA polymerase and T7 transcriptional regulatory signals was developed. The prokaryotic RNA made in mammalian cells by this system, however, was not efficiently capped. Since the 5′ cap structure is important for stability and translatability, this presented a severe problem. Stability was achieved by using DNA encoding the 5′ end of a T7 transcript with potential to form a stem-loop structure. Translatability was obtained by employing the untranslated leader sequence of encephalomyocarditis virus, which confers cap-independent ribosome binding. With the newly modified system, after 24 hours of infection the recombinant protein comprises approximately 10% of the total cell protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00306-08 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. L. Buller	Expert	LVD, NIAID
Others:	C. Duarte	Bio. Lab. Tech. (Micro)	LVD, NIAID
	G. Palumbo	IRTA Fellow	LVD, NIAID

COOPERATING UNITS *(if any)* G. Shearer and A. Singer, Cancer Immunology Branch, NCI; T. Frederickson, U. of Connecticut; D. Pickup, Duke University; L. McIntyre, Vector Lab; J. Sechler, LCI, NIAID; D. Hruby and S. Child, Oregon State U.

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Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project, we focused our studies on the genetic basis both of orthopoxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute toward the development of safe, effective recombinant vaccinia virus vaccines for animal and human use. A deletion mutant of cowpox virus has been constructed which lacks a functional gene for a 38 kDa protein involved in the generation of a hemorrhagic pock on the chorioallantoic membrane of the chicken embryo. The results to date suggest that the 38 kDa protein directly or indirectly inhibits the generation of chemotactic molecules which are elicited during virus replication in the CAM or, alternatively, blocks the interaction of these molecules with cells of the inflammatory response. A second orthopoxvirus mutant, which lacked a functional gene encoding ribonucleotide reductase, was shown to be mildly attenuated when injected into BALB/c mice by the intracranial route of inoculation. Using the cross-intercross system, the A strain of mouse (disease susceptible) was crossed with a disease resistant C57BL/6J mouse in order to generate a mouse strain A.B6-Rmp (resistance to mousepox) congenic for the resistance loci. This will aid in studying the non-H2 innate resistance genes important in resistance to disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00307-08 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	B.-Y. Ahn	Visiting Fellow	LVD, NIAID
	B. Amegadzie	Visiting Fellow	LVD, NIAID
	C. J. Baldick	Special Volunteer	LVD, NIAID
	P. Guo	Visiting Associate	LVD, NIAID
	J. Keck	NCR Fellow	LVD, NIAID
	C. Wright	Staff Fellow	LVD, NIAID

COOPERATING UNITS *(if any)* E. G. Niles, SUNY, Buffalo, NY

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL.	OTHER
5.7	4.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Vaccinia virus has a genome of about 185,000 base pairs that encodes approximately 200 polypeptides. These genes are expressed within the cytoplasm in a coordinated fashion, so that some polypeptides are made before, and others after, DNA replication. Enzymes and factors needed for early transcription are packaged within the infectious particle, while those needed for late transcription are present in the cytoplasm of infected cells. The aim of this project is to determine the mechanisms regulating transcription.

Progress in understanding early and late transcription has been made this past year. Extensive mutagenesis of an early promoter indicated that it consists of three regions: a 15 base pair critical region in which most single nucleotide substitutions have a major effect on expression, separated by 11 base pairs of a less-critical T-rich sequence from a 5 to 7 base pair region within which initiation with a purine usually occurs. Additional mutagenesis studies indicated that late promoters may also be considered in terms of three regions: a T- or A-rich upstream sequence of about 20 base pairs, a spacer region of 6 base pairs, and a highly conserved TAAATG sequence which includes the RNA start site. Previous studies indicated that a single factor, in addition to RNA polymerase, present in virus particles, was required for *in vitro* transcription of early genes. This past year, we found evidence for three factors present in the cytoplasm of infected cells that are needed for *in vitro* transcription of late genes. Genetic studies indicated that the vaccinia virus topoisomerase gene is essential, and the gene encoding the small subunit of the capping enzyme was located.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00416-06 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recombinant Vaccines against Retroviruses Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	S. Chakrabarti	Visiting Associate	LVD, NIAID
	R. Doms	IRTA Fellow	LVD, NIAID
	P. Earl	Microbiologist	LVD, NIAID
	V. Karacostas	Special Volunteer	LVD, NIAID
	R. Padmanabhan	IPA	LVD, NIAID

COOPERATING UNITS (if any) LIR, NIAID; LI, NIAID; Metabolism Branch, NCI; LTCB, NCI; LCMS, NCI; Massachusetts General Hospital, Immuno AG; Molecular Vaccines

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	3.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human immunodeficiency virus (HIV) has been identified as the etiological agent of acquired immunodeficiency syndrome (AIDS). At present, there is no effective vaccine against this disease. The object of this project is to characterize HIV antigens, determine the targets of humoral and cell-mediated immunity, and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing HIV genetic information. These viruses have been used as live vaccines to immunize animals, to synthesize HIV proteins in tissue culture, and to make targets for cytotoxic T cells. During this past year, we have succeeded in further characterizing the envelope proteins of HIV-1 and HIV-2. Both proteins were shown to exist in oligomeric forms that might enhance their immunogenicity and binding to the CD4 receptor on lymphocytes. The *gag-pol* genes of HIV-1 were expressed and the proteins were processed and shown to assemble into particles that budded from the plasma membrane. Such particles represent a novel noninfectious form of HIV that might have potential as a vaccine. Further work on the targets of cytotoxic T cells has led to the definition of specific epitopes on the envelope and reverse transcriptase proteins.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00443-05 LVD

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vaccinia Virus Growth Factor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. L. Buller	Expert	LVD, NIAID
Others:	B. Moss	Chief	LVD, NIAID
	C. Duarte	Bio. Lab. Tech. (Micro)	LVD, NIAID

COOPERATING UNITS *(if any)* T. Frederickson, Univ. of Connecticut; G. McFadden, Univ. of Alberta

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00445-05 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. D. Challberg	Senior Staff Fellow	LVD, NIAID
Others:	P. D. Olivo	Medical Staff Fellow	LVD, NIAID
	J. Gottlieb	IRTA Fellow	LVD, NIAID
	G. Sherman	IRTA Fellow	LVD, NIAID

COOPERATING UNITS *(if any)* Department of Biochemistry, Stanford Medical School

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
3.83	2.83	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying this process with purified proteins. We have previously found seven viral genes which are both necessary and sufficient to carry out authentic origin-dependent DNA replication. Ongoing biochemical analyses in several laboratories support the idea that the products of these seven genes all participate directly in viral DNA synthesis. Our recent studies have concentrated on three of these proteins: UL9, UL42, and UL30.

The HSV DNA polymerase purified from infected HeLa cells consists of a stable complex of at least two polypeptides: UL30, the catalytic subunit, and UL42. We have purified the catalytic subunit free of UL42 using a recombinant baculovirus expressing UL30. Although the purified catalytic subunit has the same specific activity as the UL30/UL42 complex using activated DNA as template, the complex is much more active on a ØX174 single-stranded DNA template primed with a short oligonucleotide. Experiments aimed at determining the mechanism by which UL42 increases the efficiency of the DNA polymerase are in progress.

We have shown that the UL9 protein binds specifically to two related sites within the origins of DNA replication. The interaction of purified UL9 with wild-type and mutated versions of the origin has been studied in some detail. Our results suggest that: *1)* the binding of UL9 to each of the two binding sites correlates well with the ability of the origin to function *in vivo;* and *2)* there is a cooperative interaction between UL9 bound at both arms of the origin palindrome. In addition to origin binding, the purified UL9 protein possesses an intrinsic 3′ to 5′ helicase activity that is capable of unwinding short duplex DNA segments in an ATP-dependent reaction. We propose that the unwinding reaction may represent a critical early step in the initiation of replication at the HSV origins of replication, generating a structure that is suitable for the assembly of the other components of the viral replication machinery at that site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00538-02 LVD

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interaction of Human Immunodeficiency Virus with the CD4 Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Berger	Expert Consultant	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	P. Ashorn	IRTA Fellow	LVD, NIAID
	T. Mizukami	Special Volunteer	LVD, NIAID

COOPERATING UNITS *(if any)* I. Pastan, V. Chaudhary, D. Fitzgerald, NCI

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.2	2.2	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD4 is a surface molecule of human helper T lymphocytes that serves as the receptor for human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS). The region of CD4 involved in binding to gp120, the external subunit of the HIV envelope glycoprotein, was analyzed in order to develop therapeutic strategies. A series of mutant CD4 genes was constructed by site-directed mutagenesis and the proteins were synthesized in mammalian cells. We found that impaired binding of secreted CD4 to soluble gp120 resulted from insertion mutations within the first domain, particularly in the region predicted to correspond to the CDR2 loop of the immunoglobulin light chain variable region. The epitopes for two HIV-blocking monoclonal antibodies also mapped to this region, whereas the epitopes for two others mapped to the second domain. Previously, we produced a recombinant molecule containing the gp120-binding region of CD4 linked to active regions of *Pseudomonas* exotoxin A and demonstrated that it selectively kills chronically-infected human T cell lines. During the past year, using a cell line that is inducible for HIV expression, we found that sensitivity to the toxin occurred only when HIV was expressed. The toxin was found to inhibit HIV spread to uninfected cells by virtue of its ability to kill infected cells. Significantly, the toxin was shown to be active against cells expressive gp120 from diverse isolates of HIV-1 as well as from HIV-2 and SIV. In addition, the toxin produced no direct cytotoxic effects or functional impairment of uninfected cells bearing surface MHC class II molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00539-02 LVD

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders*)
Virus-Cell Interactions, Viral Pathogenesis, and Host Immunity

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	S. Isaacs	Medical Staff Fellow	LVD, NIAID
	G. Kotwal	Visiting Associate	LVD, NIAID
	R. Blasco	Visiting Associate	LVD, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
2.7	2.5	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type Do not exceed the space provided*)

Vaccinia virus contains a large number of genes that are not essential for replication in tissue culture. The conservation of these genes in vaccinia virus and other members of the orthopoxvirus genus. however, signifies that the protein products have an important role in virus-host interactions. We have given the name virokine to one class of such proteins that are secreted from infected cells. The first member of this class is the vaccinia virus growth factor, VGF. We have identified two additional members. One is a 35,000 molecular weight protein that has both structural and functional similarities to the human complement 4b binding protein. The viral protein is able to block the classical complement pathway *in vitro* and may provide defense against the host immune system. Another protein of this class has a molecular weight of 14,000. Although the function of the protein is unknown, deletion of the gene attenuates the pathogenicity of vaccinia virus for mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00540-02 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Human Herpesviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Frenkel	Supervisory Microbiologist	LVD, NIAID
Others:	E. Schirmer	Biologist	LVD, NIAID
	G. Katsafanas	Biologist	LVD, NIAID
	B. Avidor	Visiting Fellow	LVD, NIAID
	L. Wyatt	Senior Staff Fellow	LVD, NIAID
	R. Danovich	NRC Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	3.4	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human herpesvirus 6 (HHV-6) is a newly identified lymphotropic herpesvirus. It was isolated from AIDS patients, from patients with lymphoproliferative disorders, from patients with exanthem subitum, and from healthy individuals. The goal of our studies is to understand virus replication and virus host-cell interactions. Our progress during the past year can be summarized as follows: *1)* the structure of the viral genome. The mapping of the viral genome and the cloning of viral DNA is underway. *2)* variations in the properties of HHV-6 isolates. Virus isolates were found to differ with respect to growth in continuous human T cell lines, antigenic reactivity, and restriction enzyme analyses. These differences could provide useful markers for functional mapping of viral genes. *3)* shutoff of host DNA replication. HHV-6 shuts off host DNA replication. The virus encodes a PAA-sensitive DNA polymerase. *4)* maturation of HHV-6 virions. Steps in virion maturation have been documented by EM analyses. *5)* the requirement for T cell activation. We have documented that HHV-6 requires T cell activation for its replication in fresh human lymphocytes. *6)* activation of HHV-6 from latency. A new virus strain (RK) was isolated following activation of latent virus from $CD4^+$ cells. *7)* the effect of IL-2 on virus replication. IL-2 exhibited a dose-dependent effect on virus replication. In the presence of high levels of recombinant IL-2, there was a significant inhibition of replication. Current studies are designed to understand these complex interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00541-02 LVD

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Folding, Assembly, and Transport of Viral Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Yewdell LVD, NIAID

Others: G. Russ
R. Doms

COOPERATING UNITS *(if any)* T. Bachi, Institute for Immunology and Virology, Zurich

LAB/BRANCH
Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.66	0.34

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The improvement of current antiviral vaccines and the development of novel vaccines depends on improving our understanding of viral attachment and fusion glycoproteins. Critical insight into the structure and function of viral glycoproteins is provided by studying their biosynthesis in virus-infected cells. We have studied the biosynthesis of influenza virus hemagglutinin (HA) and vesicular stomatitis virus (VSV) G protein. In addition to their intrinsic interest, these extensively characterized proteins serve as models to elucidate general features of viral glycoprotein structure and function.

Our work is focused on the effect of brefeldin A (BFA) on glycoprotein assembly and transport. BFA is a fungal metabolite that blocks the transport of newly synthesized proteins to the plasma membrane. In agreement with previous observations, we found that HA and G synthesized in the presence of BFA are localized to the endoplasmic reticulum (ER) of virus-infected cells. Surprisingly, despite the ER localization of these proteins, their N-linked oligosaccharides are modified by enzymes associated with the *cis*- and medial-Golgi complex (GC), but not by *trans*-GC-associated enzymes. Using a number of independent approaches, our results indicate that these paradoxical findings have a relatively simple, if surprising, explanation: BFA reversibly induces the redistribution of both resident and itinerant *cis*- and medial-GC proteins to the ER. We have also determined that BFA prevents the export of itinerant proteins from the *trans*-GC to the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00542-02 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Viral Proteins for T Cell Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Bennink	LVD, NIAID
Others:	J. Cox L. Eisenlohr J. Yewdell	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2.66	0.34

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cytotoxic T lymphocytes (CTLs) play an important role in eradicating intracellular pathogens and cancer cells. It is established to various degrees of certainty that *1)* epitopes recognized by CTL can be substituted by synthetic oligopeptides comprising as few as five residues; *2)* regions of proteins recognized by CTLs are bound to a groove present at the tip of MHC class I molecules; and *3)* nonfragmented proteins must be delivered to the cytosol of antigen-presenting cells for presentation to CTLs to occur.

In the past year, we have focused our efforts on defining the intracellular compartments in which the association of foreign antigens with class I MHC molecules occur. Our studies have utilized CTLs specific for influenza and vaccinia virus. Our findings indicate that synthetic oligopeptides containing CTL epitopes associate with class I molecules at the cell surface, while determinants processed from intact proteins present in the cytosol associate with class I molecules in an intracellular compartment, most likely the endoplasmic reticulum (ER). This conclusion is based on three independent lines of evidence. First, incubation of H-2^k antigen-presenting cells with protein synthesis inhibitors for 12 to 16 hours blocks the subsequent presentation of two K^k-restricted antigens derived from noninfectious influenza virus, but not a D^k-restricted antigen or a K^k-restricted synthetic oligopeptide. This effect parallels the difference between the half-times of the transport of K^k and D^k class I molecules from the ER. Second, presentation of influenza virus and vaccinia virus antigens to CTLs is completely abrogated by brefeldin A, a compound that blocks the intracellular transport of newly synthesized class I molecules. Brefeldin A does not block the presentation of oligopeptide determinants. Third, presentation of vaccinia virus antigens is inhibited by the co-expression of an adenovirus protein (E19) that binds class I MHC molecules in the ER and prevents their intracellular transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00563-01 LVD

PERIOD COVERED

October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Production and Characterization of gp160-Specific Monoclonal Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Yewdell LVD, NIAID

Others: J. Bennink
F. Esquivel

COOPERATING UNITS (if any) P. Johnson, Georgetown University, 12441 Parklawn Drive, Rockville, MD 20852

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2	1.66	0.34

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The envelope glycoprotein of human immunodeficiency virus (HIV) plays an essential role in the initiation of HIV infection. Glycoprotein-specific antibodies neutralize viral infectivity by preventing either viral attachment, fusion, or both, and provide protection against HIV infection *in vitro*. Induction of glycoprotein-specific antibodies is a major strategy for the development of anti-HIV vaccines.

To produce vaccines that induce antibodies capable of neutralizing the widely diverse glycoproteins present in different HIV isolates, it is essential to understand the antigenic structure of the HIV glycoprotein. Attempts to produce glycoprotein-specific mAbs have met with limited success. In this project, we are attempting to produce glycoprotein-specific mAvs using *in vitro* stimulation of splenocytes from primed or naive mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00564-01 LVD

PERIOD COVERED
October 1, 1988 to September 30 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Development of a Parvoviral Vector that Regulates Gene Expression.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name. title, laboratory and institute affiliation)*

P.I:	J.A. Rose	Section Head	LVD, NIAID
Others:	S. Chatterjee	Visiting Associate	LVD, NIAID
	K. Wong	Medical Staff Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This study was designed to develop an adeno-associated virus (AAV)-based vector that would down regulate expression of specific genes upon appropriate induction. The systems chosen for study include the downregulation of certain early viral gene products which are absolutely necessary for productive viral infection. A series of such AAV-based vectors have been created in which the endogenous AAV promoters and coding sequences were replaced with a polylinker and several strong constitutive and inducible heterologous promoters. An encapsidation system has been developed in order to produce high titres of infectious recombinant virus. Transduction of adenovirus type 5 (Ad5) transformed 293 cells with a vector designed to abrogate Ad5 E1A production results in a shut off of E1A synthesis. The growth characteristics and morphology of these cells are altered. The effect on virus production is currently being measured. Methods used for this study include: gene cloning, virus production, transfection of cells, radio-immunoprecipitation, immunofluorescence, RNA and DNA hybridization techniques.

LMSF

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1989 Annual Report
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1988, to September 30, 1989

RESEARCH HIGHLIGHTS

LMSF scientists focus on relationships between disease-causing germs and their infected eukaryotic hosts, with special attention to the microbial surface constituents involved in pathogenicity and virulence. General goals of this work are to gain understanding of infectious mechanisms and disease production and to formulate experimental bases for vaccine development and for new diagnostic methods. During FY 1989, LMSF scientists have directed their attention to *Neisseria gonorrhoeae, Chlamydia trachomatis, Borrelia burgdorferi, Rickettsia rickettsii*, group B streptococci, and human immunodeficiency virus (HIV).

***Chlamydia trachomatis* and *C. psittaci*:** LMSF scientists in the Chlamydial Diseases Section under the aegis of Harlan Caldwell made several observations that provide new understanding regarding the pathogenesis of chlamydial infections and their sequelae; they also give expanded insight into prevention of chlamydial diseases. Rick Morrison sequenced the chlamydial gene encoding a 57-kDa protein that elicits delayed ocular hypersensitivity in guinea pigs recovered from primary chlamydial infections. The 57-kDa chlamydial component is a "heat-shock" protein whose analogues occur in all eukaryotic and prokaryotic cells. It is encoded by one element of a two gene operon (*hyp*A and *hyp*B), analogous to *gro*ES and *gro*EL of *Escherichia coli*. The recombinant *hyp*B product elicits ocular hypersensitivity in previously-infected, immune guinea pigs. These findings strongly support the notion that post-chlamydia infection sequelae, including trachoma and perhaps salpingitis, are mainly hypersensitivity responses to *hyp*B by animals that may also have protective immunity to infection by homologous serovars of chlamydiae. Morrison and Nancy Watkins found that chlamydiae whose intracellular growth *in vivo* is penicillin-inhibited continue to elaborate *hyp*B; that observation suggests that "inappropriate" penicillin therapy of chlamydia infections might provoke deleterious hypersensitivity and inflammatory responses in ocular and genital loci. An extensive series of studies by Caldwell, You-Xun Zhang, and Ying Yuan defined structurally/ antigenically variable and constant portions of the major outer membrane proteins (MOMPs) in all the 15 different chlamydial serovars. They also fine-mapped those oligopeptide stretches of MOMP that elicit production of neutralizing antibodies and, in addition, they identified portions of MOMP that function as T-helper cell antigens to prime B-cells of mice to produce IgG antibodies directed toward MOMP. Dr. Zhang received an NIH Merit Award for these and previous structural and immunological investigations on MOMP. Additional studies by Nancy Watkins and Hua Su identified those portions of MOMP involved in attachment of chlamydiae to cultured cells. These findings form the bases for constructing infectious bacterial (AroE *Salmonella typhimurium*) and poliovirus (Sabin 1) vectors carrying selected recombinant chlamydial MOMP gene segments. These recombinant vectors should infect the gastrointestinal mucosa to immunize the animal host with recombinant chlamydial gene products, and they have been constructed by Zhang and Scott Manning in collaboration (on poliovirus) with J. Almond, University of Reading, United Kingdom, with whom Manning is working for the summer of '89. The recombinant chlamydial vaccines are to be evaluated soon in nonhuman primates. For these and previous contributions, Dr. Caldwell received the NIH Director's Award.

Spotted Fever Rickettsiae: LMSF scientists continue to study genes encoding two outer membrane components of spotted fever rickettsiae. Earlier results indicated that *E. coli* carrying recombinant plasmids with cloned *R. rickettsii* DNA fragments that encode either of two surface proteins (155 kDa and 120 kDa) can immunize guinea pigs against challenge with viable spotted fever organisms. For that work, Dr. Greg McDonald received a PHS Special Recognition Award. Current studies by McDonald show that one of these products (120 kDa) can be purified by extracting recombinant *E. coli* with octyl-glucopyranoside (OGP). Immunization of mice with the OGP purified 120-kDa component causes "seroconversion" and protection against lethal challenge, but immunized guinea pigs exhibit limited protection. The sequences of the 120-kDa protein-encoding genes for two *R. rickettsia* strains of differing virulence were determined by Bob Gilmore; although these surface proteins carry unique epitopes, their primary structures are identical, suggesting that their unique epitopes are related to post-translational modifications. Expression of both these proteins by

R. rickettsii during its intracellular residence and multiplication is being explored by Paul Policastro. Vish Vishwanath cloned a gene from *R. conorii* (Boutonneuse fever agent) that encodes this strain's analogue to 155 kDa of *R. rickettsii.* Immunization of guinea pigs with a lysate of *E. coli* carrying the recombinant *R. conorii* gene protects them against infectious systemic challenge with *R. rickettsii* and against cutaneous challenge with homologous *R. conorii.*

Immunoglobulin Biology: Seth Pincus has devised an immunotoxin composed of ricin A chain coupled to an anti-gp120 (HIV) monoclonal antibody; this immunotoxin specifically killed HIV infected cells and limited the spread of HIV infection in tissue culture. Pincus also studies the idiotypic network of antibody response, and has recently shown that antibody responses are impaired at all levels in transgenic mice which constitutively over-express IgM representing the first antibody species (ab1) of the network. Use of monoclonal antibodies alone and in combination with interleukins is being explored by Pincus for treatment of sepsis due to group B streptococci. Carol Horgan has used recombinant DNA techniques to engineer antibodies whose heavy chains contain murine variable portions and human constant regions; other immunoglobulin constructs of Horgan's have hinge regions modified by *in vitro* mutagenesis to examine their structure and function relationships.

Lyme Disease Spirochetes: Patty Rosa developed a sensitive, specific assay for *Borrelia burgdorferi* based on the polymerase chain reaction (PCR); her assay can detect <50 copies of the *B. burgdorferi* genome. A small number of spirochetes isolated in Europe are not detected by this assay, and their differences are being explored. PCR appears to be very useful for detecting and quantifying spirochetes in arthropod vectors, infected animal hosts, and human clinical specimens including tissue sections.

Neisseria gonorrhoeae: Work on *Neisseria gonorrhoeae* continues to focus on genetic control of two surface components--pili and outer membrane protein II (P.II)--that are related to the bacterium's pathogenicity and virulence. John Swanson showed that pilus phase and antigenic changes with concomitant recombinational alterations of the expressed pilin gene occur in cells which are unable to undergo DNA-transformation. His and others studies show that a broad variety of genetic events-- both inter and intragenomic--are involved in changing pilus expression. Molecular genetic mechanisms by which pilus gene changes occur are being studied by Stuart Hill in pilus- organisms and their pilus+ progeny. His data document the importance of oligonucleotide repeats in the pilin gene on its sequence alterations, and they demonstrate influences of donor/recipient gene sequences on DNA-transformation of pilin genes. Kolari Bhat cloned and sequenced all 11 genetic loci responsible for encoding the repertoire of P.II species in the strain previously used for human challenge studies. His sequence information is being correlated with mRNA and protein data to designate which P.II is expressed from each genetic locus and to define any recombinational exchanges that occur between the genome's multiple P.II-encoding loci. Influence of P.II gene nucleotide sequence on regulation of polypeptide synthesis has been examined by Bob Belland; he finds that slipped-strand mispairing, ribosomal slippage, and H-DNA formation, all involving the leader-encoding portions of P.II genes, correlate with changes in on/off P.II expression.

ADMINISTRATIVE REPORT

Personnel changes in LMSF during the past year included departure of Dr. Greg McDonald to a faculty position at the University of Missouri Medical Center. Technical staff changes include retirement of Mona Johnston and acquisition of Dan Hogan. Summer students in LMSF include returnees Mike Jasumback and Kathy Wells (Carroll College) along with newcomers Martin Balish (University of Wisconsin), and Denise Provost (Idaho State University). An active seminar schedule invited the following speakers: Marv Reitz (NCI), Barry Cole (Univ. Utah), Wolfgang Strittmatter (Max-Planck, Berlin), Bob Inman (Toronto, ON), Tom Shinnick (CDC), Hank Seifert (Northwestern Univ.), Burt Anderson (CDC), Ron Ogata (MBI, La Jolla), and Douglas Berg (Wash. U, St. Louis). Several prospective LMSF staff members visited RML and gave seminars, as follows: Kit Tilly (NCI), Jim Bardwell (NCI), Carol Gibbs (Max-Planck, Tübingen), and Jack Heinemann (IMB, Univ. Oregon).

HONORS AND AWARDS

NIH Honor Awards:

H. Caldwell - NIH Director's Award

G. McDonald - PHS Special Recognition Award

Y-X. Zhang - NIH Merit Award

Journal Editorial Boards:

J. Swanson - Infection and Immunity

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Cell, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Rheumatology, Microbial Pathogenesis, Proceedings of the National Academy of Sciences USA, Science, and Sexually Transmitted Diseases.

Professional Posts:

O. Barrera - Promotion Advisory Committee, NIAID

H. Caldwell - Ad Hoc Member, Microbiology and Infectious Diseases Research Committee, NIAID
Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

Invited Lectures and Participation in Meetings and Symposia:

R. Belland - Sixth International Pathogenic Neisseria Conference, Pine Mountain, Georgia
University of Utah, Salt Lake City, Utah

H. Caldwell - American Society for Microbiology, New Orleans, Louisiana

S. Hill - Sixth International Pathogenic Neisseria Conference, Pine Mountain, Georgia

C. Horgan - Lake Tahoe Symposium: Advances in Recombinant DNA Technology; Genetic Engineering and the Immune System, Incline Village, Nevada

S. Manning - American Society for Microbiology, New Orleans, Louisiana

G. McDonald - University of Missouri, Columbia, Missouri
Purdue University, West Lafayette, Indiana

R. Morrison - Federation of American Societies for Experimental Biology, New Orleans, Louisiana
American Society for Microbiology, New Orleans, Louisiana
NIH Workshop on Microbial Determinants of Virulence and Host Response
University of Florida, Gainesville, Florida

S. Pincus - UCLA Symposium: Human Retroviruses, Tamarron, Colorado
Centers for Disease Control, Atlanta, Georgia
Arthritis Foundation Fellowship Committee, Atlanta, Georgia

P. Rosa - NIAID-NIAMSD Scientific Workshop on Lyme Disease, Bethesda, Maryland

H. Su - American Society for Microbiology, New Orleans, Louisiana

J. Swanson - Sixth International Pathogenic Neisseria Conference, Pine Mountain, Georgia
NIH Workshop on Microbial Determinants of Virulence and Host Response University of Florida, Gainesville, Florida
Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina
NIH Symposium on Biomedical Careers for American Indians and Alaska Natives, Phoenix, Arizona

S. Vishwanath - American Society for Microbiology, New Orleans, Louisiana
Department of Pathology, University of Texas Medical Branch, Galveston, Texas
Department of Microbiology, Medical College of Ohio, Toledo, Ohio
Division of Infectious Diseases, Case Western University School of Medicine, Cleveland,Ohio

N. Watkins - American Society for Microbiology, New Orleans, Louisiana
Department of Pathology, University of Texas Medical Branch, Galveston, Texas
Department of Microbiology, Medical College of Ohio, Toledo, Ohio
Division of Infectious Diseases, Case Western University School of Medicine, Cleveland, Ohio

Y. Yuan - American Society for Microbiology, New Orleans, Louisiana

Y. Zhang - American Society for Microbiology, New Orleans, Louisiana

Other Activities:

H. Caldwell - Reviewed research grants for Medical Research Council, Canada, National Science Foundation, Washington, DC, and The Edna McConnell Clark Foundation, New York, NY

S. Pincus - Reviewed research grants for Arthritis Foundation.

J. Swanson - Reviewed research grants for National Science Foundation, Washington, DC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00193-10 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J. Swanson	Chief	LMSF, NIAID
Others:	K. Bhat	Senior Staff Fellow	LMSF, NIAID
	R. Belland	Visiting Associate	LMSF, NIAID
	S. Hill	IRTA Fellow	LMSF, NIAID
	O. Barrera	Microbiologist	LMSF, NIAID
	S. Morrison	Microbiologist	LMSF, NIAID
	D. Corwin	Bio. Lab. Tech.	LPB, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.6	3.9	2.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The surface components of gonococci (Gc) dictate this bacterium's pathogenic personality. Pili appear to initiate and maintain adherence of Gc to a host mucosae thereby promoting colonization and infection. This is vividly reflected by virulence of pilus+ and avirulence of pilus- Gc in human volunteers. Expression of outer membrane protein II components is highly favored for gonococci *in vivo*, as demonstrated in volunteers' infections. Previous work from this laboratory focused on structural variation among pili and protein II elaborated by Gc recovered from experimental human infections. Current work is directed toward illuminating the molecular mechanisms whereby gonococci control expression and variation of these pilus and outer membrane protein II (P.II) surface constituents. Experiments during the past year have demonstrated that slipped-strand mispairing, ribosomal slippage, and DNA triple strand formation play key roles in P.II expression from their corresponding *opa* genes (*opa* for colony opacity). The entire *opa* repertoire (11 different genes) from one strain has been cloned and sequenced. The P.II product of each *opa* has been defined by *in vitro* expression and by identifying its mRNA transcript. The genetic events that generate pilus diversity are being studied by examining an unusual pilin gene whose "missense" pilin polypeptide forms no pili and its pilus+ revertants. The role of intragenomic exchanges as it effects pilus variation has been evaluated in wild-type and in mutant gonococci that are unable to take-up DNA. These studies provide considerable new insight into two distinct, seemingly simple, yet highly versatile genetic systems used by gonococci to produce and change their surface proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00216-09 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunology of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. D. Caldwell	Research Microbiologist	LMSF, NIAID
Others:	Y.-X. Zhang	Visiting Associate	LMSF, NIAID
	H. Su	Visiting Fellow	LMSF, NIAID
	Y. Yuan	Visiting Fellow	LMSF, NIAID
	D. S. Manning	IRTA Fellow	LMSF, NIAID
	S. Stewart	Microbiologist	LMSF, NIAID
	J. Simmons	Bio. Lab. Tech. (Micro.)	LMSF, NIAID

COOPERATING UNITS *(if any)*

Jeffrey Almond,Department of Microbiology and Immunology, University of Reading, Reading, U.K.;and Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

Chlamydial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.05	4.55	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The antigenic properties of the chlamydial major outer membrane protein (MOMP) are being defined at the molecular level. The findings of these studies are being used to develop novel infectious recombinant vaccine vectors which are targeted to generate chlamydial specific immunity at mucosal surfaces. Studies were carried out to identify and characterize (1) MOMP B-cell neutralizing determinants, (2) MOMP T-helper cell determinants which are capable of directing antibody responses specific to MOMP B-cell epitopes and, (3) the genetic construction of attenuated infectious enteric vaccine vectors expressing immunogenic recombinant B- and T-cell epitopes of the MOMP.

The MOMP gene(s) of four *C. trachomatis* serotypes were cloned, sequenced, and B-cell antigenic determinants identified by lambda gt11 and synthetic peptide epitope mapping methodologies. Sequence analysis showed the MOMP genes to be highly conserved being interspersed by four short symmetrically spaced variable domains (VD). Immunodominant serotype-specific neutralizing sites were mapped to contiguous sequences (8-12 residues) located in VD I or VD II of the MOMP. More broadly reactive neutralizing determinants were mapped to linear sequences located in VD IV.

Immunization of A/J mice with MOMP elicited T-lymphocyte populations capable of recognizing synthetic peptides corresponding to MOMP amino acid residues 16-40, 91-115, 151-175, and 331-355. We have used an *in vivo* assay to directly determine if these synthetic peptides were MOMP specific T-helper cell antigens. Synthetic peptides corresponding to amino acid residues 151-175 and 331-335 were capable of priming mice to elicit IgG antibodies reactive with B-cell neutralizing sites located in VD I and VD IV.

The Sabin 1 strain of poliovirus has been engineered to express immunogenic B-cell neutralizing MOMP epitopes as VP1 antigenic chimeras. Recombinant poliovirus chimeras expressing MOMP serotype-specific epitopes elicited serotype-specific neutralizing antibodies in rabbits. The immunogenicity and vaccine efficacy of poliovirus-MOMP chimeras are currently being evaluated in a non-human primate model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00441-05 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rickettsia rickettsii Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. A. McDonald	Senior Staff Fellow	LMSF, NIAID
Others:	R. D. Gilmore	IRTA Fellow	LMSF, NIAID
	S. Vishwanath	Senior Staff Fellow	LMSF, NIAID
	P. F. Policastro	Senior Staff Fellow	LMSF, NIAID
	R. E. Mann	Bio. Lab. Tech.	LMSF, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.8	3.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in the characterization of two proteins of *Rickettsia rickettsii* and *R. conorii*. The two *R. rickettsii* proteins (120 and 155 kDa) are immunogenic, have been implicated as virulence factors, and may be subunit vaccine candidates. The genes encoding these proteins have been cloned and expressed in *Escherichia coli*; the same has been done with the gene encoding a *R. conorii* protein (200 kDa) which is analogous to the *R. rickettsii* 155-kDa protein. Fractions of sonic lysates of *E. coli* expressing the 120-kDa protein were used to immunize mice against challenge with viable *R. rickettsii* and guinea pigs against experimental Rocky Mountain spotted fever (RMSF); immunity and seroconversion were elicited in mice, but the immunity produced in guinea pigs was relatively low. Sonic lysates of *E. coli* expressing the 200-kDa *R. conorii* protein were used to vaccinate guinea pigs against challenge with both *R. rickettsii* and *R. conorii*; good immunity and seroconversion were produced to both organisms. The gene encoding the 120-kDa protein was initially cloned from a virulent strain and was found to hybridize to DNA from the avirulent HLP strain; the HLP gene was subsequently cloned. Sequence analyses of both genes revealed that they are identical. The gene encoding the 155-kDa protein is being sequenced in collaboration with researchers at the C.D.C. Six thousand bases of open reading have been sequenced and a 3' stop codon has been found, but the 5' end of the gene has not been unequivocally identified. Temporal regulation of transcription of the 120 and 155 genes is being studied. Vero cells were infected with *R. rickettsii* and RNA was isolated at different times post-infection. The RNA was subjected to northern blot analyses and hybridization with the 120 and 155 genes. Transcripts from the 120 gene were detected while none were identified for the 155 gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00516-02 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Immunoglobulin Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	S. H. Pincus	Expert	LMSF, NIAID
Others:	C. J. Horgan	Senior Staff Fellow	LMSF, NIAID
	R. L. Cole	Chemist	LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER.
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this work is to understand the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy.

A. Therapeutic Systems. 1. Antibodies to surface antigens of group-B streptococci have been demonstrated to have protective efficacy in a model of neonatal sepsis. We have shown that the interleukin GM-CSF (granulocyte monocyte colony stimulating factor) has protective efficacy alone, and additive efficacy when applied in combination with antibody. 2. The *in vitro* efficacy of anti-HIV envelope (gp 120) monoclonal antibodies has been tested. Antibody coupled to ricin A-chain is highly lethal to infected targets while sparing uninfected cells. We have used the immunotoxin to select for biological variants of HIV. We have begun in vivo studies to test the efficacy of antibody based therapies of AIDS.

B. Genetically Engineered Antibodies. Vectors have been prepared carrying human mu, gamma-1, and gamma-4 constant region genes in an enhancer containing expression vector. Variable region genes from anti-(T,G)-A--L antibodies have been inserted. DNA has been transfected into cell lines by electroporation and expressed as functional antibody. Variant hinge region genes are being constructed. These will be used to study the role of the antibody hinge in Fc-mediated effector function.

C. Idiotypy. The idiotypic network induced in C57BL/10 mice by immunization with the synthetic polypeptide (Tyr, Glu)-Ala--Lys is being explored. Transgenic mice that over-express the ab 1 heavy chain have been bred. Studies of their immune response to four different antigens have been performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00518-02 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigenic Mapping of Chlamydial Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. G. Watkins	Senior Staff Fellow	LMSF, NIAID
Others:	H. Su	Visiting Fellow	LMSF, NIAID
	H. D. Caldwell	Research Microbiologist	LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

Chlamydial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.35	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major outer membrane protein (MOMP) of *Chlamydia trachomatis* has been identified as a possible immunogen for vaccine development. The objectives of this project are to identify amino acid sequences of MOMP which are surface accessible to immunoglobulin and to determine if these sequences are involved in chlamydiae-host cell attachment. Peptides containing sequences of three of the four variable domains (VD I, VD II, and VD III) have been synthesized. These peptides have been used to define surface accessible epitopes of serovars of the B and intermediate serogroup. VD II and VD IV or the MOMP of serovars in the B serogroup are surface accessible, whereas VD I and VD II are not accessible to antibody at the cell surface. The MOMP of the intermediate serogroup contains a surface accessible VD I and VD IV. Trypsin cleavage of VD II and VD IV of the MOMP of serovar B on the surface of viable EBs results in loss of binding of chlamydiae to HeLa 229 cells, suggesting a role for MOMP in interactions of chlamydiae with host cells. Synthetic peptides corresponding to VD II and VD IV do not inhibit the binding of chlamydiae to host cells indicating that chlamydiae-host cell binding is dependent upon more than the primary sequences in these variable domains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 AI 00519-02 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Chlamydial Antigens: Role in Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. P. Morrison	Senior Staff Fellow	LMSF, NIAID
Others:	H. D. Caldwell	Research Microbiologist	LMSF, NIAID
	R. J. Belland	Visiting Associate	LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

Chlamydial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.45	1.2	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chlamydia trachomatis infection of humans is commonly a localized inflammation that can progress to serious complications such as infertility, blindness and perhaps arthritis. The pathogenic process(es) that cause these serious sequelae are thought to be immunological. A 57-kDa protein that is common among *Chlamydia* elicits ocular inflammation when introduced onto the conjunctivae of guinea pigs previously sensitized by chlamydial infection. The inflammatory response is characterized by a submucosal infiltrate of mononuclear macrophages and lymphocytes (DTH response), and is indistinguishable from that observed in the conjunctivae of individuals with trachoma and in the fallopian tubes of women rendered infertile as the result of chlamydial induced salpingitis. To more thoroughly characterize this chlamydial antigen, we cloned and sequenced its gene from a *C. psittaci* strain. Immunoaffinity-purified recombinant 57-kDa protein elicited an ocular DTH response similar to that produced by the 57-kDa antigen isolated from chlamydiae. Sequencing of the recombinant clone identified two open reading frames that encode polypeptides of 11.2 kDa and 58.1 kDa and are co-transcribed. We have termed the operon *hyp* (for hypersensitivity); *hypA* encodes the 11.2-kDa polypeptide and *hypB* encodes the 58.1-kDa polypeptide. These two polypeptides show striking homology with the GroE heat-shock proteins of *E. coli* (GroES and GroEL). The *hyp* operon is highly conserved among *Chlamydia* as demonstrated by complete nucleotide identity between the *hyp* operons of both *C. psittaci* and *C. trachomatis* strains. Thus, the 57-kDa chlamydial protein, previously implicated as mediating a deleterious immunologic response to chlamydial infections, is a stress-induced protein similar to those that occur universally in both prokaryotic and eukaryotic organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 AI 00549-01 LMSF

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Infection with the Lyme Disease Spirochete, *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. A. Rosa	Senior Staff Fellow	LMSF, NIAID
Others:	D. M. Hogan	Microbiologist	LMSF, NIAID

COOPERATING UNITS *(if any)*

T. G. Schwan, NIAID, RML, Laboratory of Pathobiology

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A specific and sensitive assay for the Lyme disease spirochete, *Borrelia burgdorferi*, was developed from a randomly cloned sequence of *B.b.* DNA using the polymerase chain reaction (PCR) technique. Current investigations into the transmission and pathogenesis of Lyme disease, as well as its diagnosis, are limited by the general inability to detect the causative agent with standard serologic techniques. This PCR assay exhibits the following characteristics:

The sensitivity of the PCR assay was determined with purified *B. burgdorferi* DNA, crude culture lysates, and formalin-fixed, paraffin-imbedded spirochetes. With all sources, the target sequence could be amplified to visually detectable levels from fewer than 50 copies of the *B. burgdorferi* genome. This degree of sensitivity is maintained in the presence of a million-fold excess of eukaryotic (human and mouse) DNA. Preliminary experiments with this PCR assay have demonstrated *B. burgdorferi* in the blood of experimentally infected mice as well as in ticks which have fed on infected mice. Hence this PCR assay is applicable to the detection of spirochetes in infected tissues.

The specificity of this PCR assay was tested with purified DNA from other members of the genus *Borrelia*. These include 4 species which cause relapsing fever, a bird spirochete, and a spirochete thought to cause epidemic bovine abortion. No amplified products were detected for any of these spirochetes, even when the PCR was continued for an additional 20 cycles. Thus there is no apparent cross-reactivity with other spirochetes in the genus. The universality of this PCR assay for the Lyme disease spirochete was tested by amplifying DNA of 8 different *B. burgdorferi* isolates from a broad geographic area, and of both human and tick origin. A specific amplified product was detected with 7 of 8 strains. Hence most strains of *B. burgdorferi* are detected with this PCR assay. We have cloned the equivalent target segment of DNA from the European strain which was not amplified and will design primers corresponding to its sequence. It is likely that these primers will permit detection of a class of European strains which are distinct from North American isolates, but conserved among themselves.

This PCR assay should greatly facilitate the accurate diagnosis of Lyme disease as well as provide a means with which to investigate the pathogenesis, transmission, and basic biology of *B. burgdorferi*.

LPB

LABORATORY OF PATHOBIOLOGY
Rocky Mountain Laboratories
Hamilton, Montana
1989 Annual Report
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ANNUAL REPORT
LABORATORY OF PATHOBIOLOGY
ROCKY MOUNTAIN LABORATORIES
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1988, to SEPTEMBER 30, 1989

The present makeup of the Laboratory of Pathobiology provides a unique and innovative mixture of basic biology, biochemistry, immunology, electron microscopy, arthropod vector biology, and molecular biology with strong interaction among its own members and with other units both inside and outside of the NIH system. This interaction together with an active visiting, seminar and guest researcher program insures that the best experimental approaches are incorporated quickly into the research programs of the laboratory. The focus of the laboratory has remained, however, on the use of modern methods of molecular biology to characterize in molecular terms critical aspects of host-pathogen interaction. The Laboratory of Pathobiology has functioned in four major experimental groups emphasizing two human pathogens - *Bordetella pertussis* and *Borrelia burgdorferi*. Recently, a third has been added - the most common cause of bacterial gastroenteritis in man - *Campylobacter jejuni*. Dr. Claude F. Garon serves as Chief of the Laboratory.

The Molecular Pathobiology Section, initially under the direction of Dr. Jerry Keith, has worked on the molecular cloning and expression of genes involved in the production of the toxic components of *Bordetella pertussis*. The S1 subunit of the pertussis toxin is an enzymatically active protein that catalyzes the transfer of ADP-ribose from NAD to guanine nucleotide regulatory proteins in eukaryotic cell membranes. The gene encoding the S1 subunit, as well as several truncated versions, has been expressed in *Escherichia coli* using vectors containing the transcriptional and translational control elements of the *lac* operon. By employing these recombinant S1 subunit constructs, the structure - function relationship of pertussis toxin, as well as important aspects of pathogenesis and immunoprotection, may be accurately assessed. Of particular significance was the finding that a region spanning 8 amino acids and located near the amino terminus was not only essential for function, but showed considerable sequence homology to similarly located sequences in subunits of cholera toxin and *E. coli* heat-labile toxin I. Furthermore, the introduction of iso-ionic single amino acid substitutions into this region yielded several mutants with significantly decreased enzymatic activity. Further biochemical and genetic studies have supported the direct role for this region in the active site of S1. All of the single substitution mutants continued to react with protective antibodies. The effects of these specific mutations on toxicity an virulence are currently being examined by introducing them into the *B. pertussis* genome using a shuttle-replacement vector.

The Immunopathology Section initially with Dr. John Munoz as Acting Head, has characterized the immunopotentiating action of small quantities of crystalline pertussis toxin (PTX). These studies have acquired added importance given the role of PTX in boosting protective antibody responses. PTX increased strikingly the protective action of vaccines made from *Bordetella pertussis* strains lacking PTX, or from strains in which a genetically modified gene for the S1 subunit of PTX was present. PTX also enhanced the protective activity of vaccines made from *B. parapertussis* and *B. bronchiseptica*, but had little effect on vaccines made from wild-type strains of *B. pertussis* known to produce PTX. The antibody response to surface antigens of *Bordetella* was also studied. It was shown that the antibodies of the IgG class are increased with all the vaccines used, the IgM is only slightly increased, and the IgA class is not increased. However, the IgE class of antibodies was noticeably increased in vaccines containing PTX or when PTX was included in the immunization with vaccines lacking PTX. It was previously shown that any modification of the gene responsible for the production of the S1 subunit of PTX, made the *B. pertussis* cells ineffective in all the immunopotentiating actions found in vaccines made from wild-type strains containing the intact toxin gene. These observations have been extended to vaccines made with *B. parapertussis* into which unmodified PTX genes, or genes modified in the S1 region or other subunits had been introduced. Vaccines made from *B. parapertussis* with the intact gene had all the immunopotentiating activities of the wild-type *B. pertussis*, including the induction of protection against intracerebral (IC) challenge. *B. parapertussis* mutants containing a modified S1 gene or a gene lacking S1 did not have any of the immunomodulating activities of wild-type *B. pertussis* cells. The activities measured were: histamine sensitization, leukocytosis promotion, enhancement of anaphylaxis, enhancement of IgE production, increase in the percentage of IgE containing cells in the spleen and the ability to protect mice from IC challenge with virulent *B. pertussis*. Vaccines made from *B. pertussis* strains are only weakly protective against IC challenge with *B. pertussis*, however, strains into which the PTX gene had been introduced became highly effective in inducing protection. As in the case of *B. pertussis* strains, the *B. parapertussis* strains containing a PTX gene in which the S1 gene had been modified were not any more protective than the carrier strain of *B. parapertussis*. If PTX was given, in addition to these weakly protective vaccines, the vaccines became highly effective. Dr. Munoz retired in December 1988.

Lyme disease is now the most common arthropod-borne disease in the United States. While originally thought to be confined to limited areas, the disease has now been found in at least 43 states and 6 continents. Members of the Laboratory have made a major commitment to an improvement in diagnostic reagents and to an understanding (in molecular terms) of how this pathogen produces its long list of clinical features. The Arthropod-borne Diseases Section under the direction of Dr. Tom Schwan has concentrated on this and other vector-borne pathogens. Recently, this section has demonstrated that continuous cultivation of the Lyme disease spirochete in modified Kelly's medium causes a reduction in the number of detectable plasmids and the loss of infectivity in the white-footed mouse. In an unpassaged culture of *Borrelia burgdorferi*, nine plasmids were present, including seven linear plasmids ranging in size from 49 to 16 kilobases (kb) and two circular plasmids of 27 and 7.6 kb. The 7.6 kb circular and 22 kb linear plasmids were no longer detectable in spirochetes noninfective in white-footed mice, suggesting that gene(s) encoding for factors responsible for infection may be present on one or more of these extrachromosomal elements. Furthermore, changes in spirochetal proteins and lipopolysaccharide-like material were observed also during early cultivation and may be related to loss of infectivity. The 7.6 kb circular plasmid present in several low passage strains of *B. burgdorferi* has been identified as a strong candidate for both regulating infection and as a marker for potentially infectious strains. This plasmid has been purified and restriction fragments of approximately 200 and 500 basepairs have been cloned, and are being tested as DNA hybridization probes.

Also participating in research on the molecular biology of the Lyme disease spirochete is the Pathobiology Section under the direction of Dr. Claude Garon. Earlier descriptions of covalently-closed, double-stranded, linear plasmids in *Borrelia burgdorferi* by members of this laboratory has led to a more careful analysis of several low passage strains by agarose gel electrophoresis and by nucleic acid electron microscopy. The plasmid pool of one isolate contained nearly 200 kb of unique DNA sequences including both linear (5.4, 19.5, 21.5, 31, 36, and 53 kb) and circular (26 kb) molecules. Since accurate size determination by agarose gel electrophoresis is not possible with an undefined mixture of linear and circular plasmids, a new method was developed to identify molecular form in these samples without band purification or electron microscopy. While all of the linear species rapidly reannealed to linear duplexes after alkaline denaturation, single-stranded circular molecules measuring twice the length of the linears could be produced by treatment with methyl mercury, glyoxal and urea prior to mounting for microscopy. Both circle formation and rapid reannealing could be prevented by pretreatment with single-strand specific nucleases. Molecules could then be compared by heteroduplex analysis. One of the linear plasmids, in addition to its covalently-closed structure, appeared to possess a unique inverted redundancy of 2.3 kb located near the molecular termini. Two prominent stem-loop structures possibly suggesting transposable elements were detected as well. Electroporation experiments have demonstrated the feasibility of developing a genetic transfer system in *B. burgdorferi*. Studies on the replication of these linear plasmids, with their terminal hairpin structures (which may resemble telomeres of eukaryotic chromosomes) are underway as well. The identification of a new, nucleic acid-containing compartment in several microorganisms (including *Borrelia burgdorferi*) together with the observation, so far only in *Neisseria gonorrhoeae*, that a new mechanism of genetic transfer involving naturally elaborated membrane blebs is possible, has opened a new area to scientific scrutiny. This possibility represents a major area of investigation for this section.

Although changes in personnel and new data have in the past and will in the future continue to bring subtle changes in research direction, the goal of the Laboratory of Pathobiology remains a multidisciplinary approach to molecular pathogenesis. Building on a long history of achievement in the area, special emphasis remains as well on the interactions of vectors and pathogens.

ADMINISTRATIVE

Dr. John Munoz was appointed scientist emeritus in the Laboratory of Pathobiology during the year. Dr. Willy Burgdorfer has continued to provide valuable support for the Arthropod-borne Diseases Section.

Guest Researchers in the Laboratory of Pathobiology have included: Dr. Stanley Falkow (Stanford University School of Medicine) and Kirsten Vadheim (Montana State University). Joining the Laboratory was Dr. Christopher W. Cluff (Emory University) as an IRTA Fellow and Dr. Witold Cieplak, Jr. (University of Texas Health Science Center at Dallas) as a Senior Staff Fellow.

Visitors/Collaborators who spent varying amounts of time in the laboratory during the past year included:

NAME	AFFILIATION
Dr. Robert Lane	University of California, Berkeley
Kirsten Vadheim	Montana State University, Bozeman, MT
Sirkka Vene	Department of Virology, Stockholm, Sweden
Skip Stowe	Provincial Laboratory of Public Health, Calgary, Alberta, Canada
Dr. Kevin Fonseca	Provincial Laboratory of Public Health, Calgary, Alberta, Canada
Dr. Carey Tanner	Stanford University and Santa Clara County Health Department, CA
Dr. Craig Green	University of Georgia, Athens
Dr. Peter Hindderson	Statens Seruminstitut, Copenhagen, Denmark
Dr. Terry D. Allen	Paterson Institute for Cancer Research, Manchester, United Kingdom
Dr. Janis Weis	University of Utah, Salt Lake City, UT
Dr. Lynita Cooksey	Colorado State University, Fort Collins
Ms. Sonya Ubico	Colorado State University, Fort Collins
Dr. Richard Sautter	Marshfield Research Clinic, WI

The Laboratory of Pathobiology was reviewed by the National Institute of Allergy and Infectious Diseases Board of Scientific Counselors in May 1989.

HONORS AND AWARDS

Dr. Tom G. Schwan

Invited speaker:

Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC); Los Angeles, CA, 24-26 October 1988.
Scientific Workshop on Lyme Disease, NIAID and NIAMSD; Bethesda, MD, 13-15 December 1988.
National Advisory Allergy and Infectious Diseases Council; Bethesda, MD, 26 January 1989.
Lyme Borreliosis Foundation meeting; Austin, TX, 4-5 May 1989.
New York Medical College, Valhalla, NY, 15-16 June 1989.
Bowman Gray School of Medicine, Wake Forest University; Winston-Salem, NC, 19-20 June 1989.
Invited member to ad hoc advisory committee to NIAID for Scientific Workshop on Lyme Disease and recommendations for future funding for Lyme disease research.
Presented papers at American Society for Microbiology meeting; New Orleans, LA, 15-18 May 1989.

Reviewed manuscripts for:

European Journal of Clinical Microbiology and Infectious Diseases
Journal of the American Medical Association
Journal of Infectious Diseases
Journal of Wildlife Diseases
New England Journal of Medicine
Public Health Reports
The Southwestern Naturalist

Dr. John J. Munoz

Reappointed as a staff affiliate of the University of Montana

Trustee of the Stella Duncan Memorial Research Fund, University of Montana

Reviewed manuscripts for Infection and Immunity

Reviewed a grant for Alberta Heritage Foundation for Medical Research

Received Scientist Emeritus position in Laboratory of Pathobiology

Received Emeritus status in the following:

American Society of Microbiology
Reticuloendothelial Society
Society for Experimental Biology and Medicine

Dr. Claude F. Garon

Served on State Board of Governors - Center of Excellence in Biotechnology. Montana Science and Technology Alliance.

Faculty Affiliate - Division of Biological Sciences. University of Montana.

Internal Advisory Committee. University of Montana Electron Microscopy Facility.

Invited Speaker:

American Society for Biochemistry and Molecular Biology, San Francisco, CA
American Society for Microbiology, New Orleans, LA
Continuing Education Series for Science Teachers on Genetic Engineering, Missoula, MT
Montana State Teachers Association Convention
Molecular Biology Seminar Series - University of Montana
Hamilton Lions Club
Presbyterian Mens Club
Hamilton High School
Florence High School

Dr. Witold Cieplak, Jr.

Invited Speaker:

Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX

Meetings Attended:

American Society for Microbiology, New Orleans, LA

Reviewed Manuscripts For:

Journal of Biological Chemistry
Infection and Immunity

Dr. Willy Burgdorfer

Invited to present lecture at the 1988 Conference of the Pacific Northwest Chapter/Medical Library Association, Bozeman, MT (October 6, 1988).

Invited to present lecture on Tick-borne spirochetoses (relapsing fevers, Lyme disease) during the 1988 Northwest Medical Laboratory Symposium, Seattle, WA (October 18-22, 1988).

Invited to present guest lecture at the Pettenkofer Institut, Munich, West Germany (November 9, 1988).

Invited speaker at the 1988 Entomological Society of American National Conference, Louisville, KY (December 4-8, 1988).

Invited to chair session "Ecology of the Lyme disease agent, *Borrelia burgdorferi*" at NIAID and NIAMSD sponsored scientific workshop on Lyme disease, Washington, D.C. (December 13-15, 1988).

Invited to speak on Lyme disease during Planning Session with NIH Director, Dr. J. B. Wyngaarden (January 30, 1989).

Invited to speak at the 42nd Annual Meeting of the Montana Society for Medical Technology, Missoula, MT (April 12-15, 1989).

Invited to present seminar on Lyme disease research at Molecular Diagnostic, Inc., West Haven, CT (April 19, 1989).

Invited to participate and present lecture at Lyme Disease Symposium at Phelps Memorial Hospital, North Tarrytown, New York, NY (April 27, 1989).

Invited to participate at Lyme Disease Workshop and Symposium, Austin, TX (May 4-6, 1989).

Speaker at Missoula Rotary Club, Missoula, MT (May 10, 1989).

Invited to present lecture at Lyme Disease Consortium, Marshfield, WI (May 18, 1989).

Invited to speak at 1989 Allergy, Immunology, and Asthma Symposium, Oshkosh, WI (May 19, 1989).

Invited speaker at Medical Arts Center Symposium, Memphis, TN (May 27, 1989).

Invited speaker at Marshfield Clinic, Marshfield, WI (June 5, 1989).

Invited speaker at Lyme Disease Symposium and Workshop, Madison, WI (June 6-7, 1989).

Participated as instructor at the 38th Acarology Summer Program, Ohio State University, Columbus, OH (June 24-30, 1989).

Invited to present lecture on Lyme disease at Ohio State Department of Health, Columbus, OH (June 24, 1989).

Invited speaker at INCDNCN meeting in Calgary, Alberta, Canada. (August 13-16, 1989.

Nominated recipient of the Bristol Award of the Infectious Diseases Society of America to be presented September 16, 1989, in Houston, TX.

Recipient of NIAID Plaque-Award in recognition of the discovery of the causative agent of Lyme disease, Recipient of the Robert Koch Gold Medal Award by the Robert Koch Foundation, Bonn, West Germany (November 7, 1988).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00071-18 LPB

PERIOD COVERED

October 1, 1988, to January 1, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Pertussigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John J. Munoz	Research Microbiologist	LPB, NIAID
OTHERS: M. G. Peacock	Microbiologist	LPN, NIAID

COOPERATING UNITS *(if any)*

Stanford University (W. J. Black), Praxis Laboratories (J. L. Cowell)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Immunopathology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.15	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Whole cell pertussis vaccine made from wild-type strains of *Bordetella pertussis* protect mice against intracerebral (IC) challenge with virulent strain. Similar vaccines made from strains that do not produce pertussis toxin (Ptx) do not protect. These Ptx deficient strains were either naturally occurring Phase III cultures or cultures developed by modifying the gene responsible for Ptx production. Nanogram doses of Ptx added to vaccines made from Ptx-deficient cells converted them into highly efficient vaccines for the protection of mice to IC challenge.

Purified surface antigens of *B. pertussis* such as filamentous hemagglutinin which do not protect mice to IC challenge with *B. pertussis* were also made effective when nanogram doses of Ptx were added. The doses of Ptx did not develop detectable antibodies to itself.

Two actions of Ptx are possibly involved in this protection-inducing effect. One is its ability to stimulate antibodies specially of the IgE class, and the other is its ability to counteract the action of epinephrine responsible for preventing increases in capillary permeability.

When Ptx was given to mice 4 hours to 4 days prior to an antiserum directed to surface antigens of *B. pertussis* it also was able to increase the protective activity of the antisera. This action is probably mainly due to the non-specific effect of Ptx which inhibits and protects the permeability-neutralizing action of epinephrine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00201-10 LPB

PERIOD COVERED
October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Characterization of Integrated Viral Genomes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Claude F. Garon	Chief	LPB, NIAID
OTHERS:		
L. L. Lubke	Biologist	LPB, NIAID
S. F. Hayes	Biol. Lab. Tech. (Micro)	LPB, NIAID
D. Corwin	Biol. Lab. Tech. (Micro)	LPB, NIAID

COOPERATING UNITS *(if any)*
LVD (Moss, LMM (Martin, Khan), LMO/NCI (T. S. Papas), CDC (Mayer)

LAB/BRANCH
Laboratory of Pathobiology

SECTION
Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.43	0.2	1.23

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Modern methods of molecular cloning and analysis allow for the detection and amplification of rare DNA sequences such as a single integrated viral segment. Molecular clones of several newly integrated retroviral genomes have been produced in either plasmid or bacteriophage cloning vehicles using recombinant DNA techniques and have been characterized using electron microscope heteroduplex and R-loop methods. Detection of sequence homology even when rearranged or interrupted by intervening cellular DNA is often accurately mappable in the electron microscope using these methods. These studies have not only shown the arrangement of integrated viral sequences within infected host cell DNA, but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells as well. These studies have recently been expanded to include a structural determination of the genetic relatedness of AIDS virus isolates from around the world. Unique inverted repeat sequences structurally resembling transposable elements have been identified in the area of retroviral integrations and molecularly cloned for further analysis. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of integrative position of flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination in eukaryotic cell systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00402-06 LPB

PERIOD COVERED
October 1, 1988, to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Cloning and Expression of *Bordetella pertussis* Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jerry M. Keith	Head	LPB, NIAID
OTHERS:	K Vadheim & S. Strachan	Guest Workers	LPB, NIAID
	M. Szulczynski & Y. Lobet	Visiting Fellows	LPB, NIAID
	K. Kaljot	NRSA Researcher	LPB, NIAID
	S. Northcutt & S. Smith	Microbiologists	LPB, NIAID
	D. Brown	Sr. Staff Fellow	LPB, NIAID
	W. Cieplak	NRC Associate	LPB, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pathobiology

SECTION
Molecular Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.95	1.55	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pertussis toxin (PTX) is a hexameric protein produced by *Bordetella pertussis* and is comprised of five different subunits termed S1 through S5. PTX exerts a diverse array of biological effects *in vitro* and *in vivo*, and is believed to occupy a central role in the pathogenesis of whooping cough or pertussis. Many of these effects appear to result from the ability of PTX to catalyze the ADP-ribosylation of guanine nucleotide-binding proteins of the adenylate cyclase complex, using NAD+ as the ADP-ribose donor. This enzymatic modification results in impaired regulation of cyclic AMP production with consequent perturbation of cell function. The ADP-ribosyltransferase activity is associated with the S1 subunit (Mr 28,000) of PTX. PTX has also been shown to be a major antigen in acellular vaccines. One of the goals of our laboratory is to study the structure-function relationships of this protein. In the light of the current discussions about the safety of pertussis vaccines, a better knowledge of the enzymatic mechanism of PTX is of particular interest because it could lead to the development of an enzymatically inactive but immunologically intact toxin, able to elicit protection against the disease without the harmful side effects.

Our most important scientific advance derived from the gene expression experiments was the discovery of a molecular approach for inactivation of pertussis toxin biological activity. Using site-specific DNA mutagenesis, the S1 subunit was modified by a single amino acid substitution. This mutation virtually eliminated enzymatic activity, yet the immunogenic protective epitope was retained.

In order to take advantage of this important finding, we have devised several methods to transfer these genetic changes into the chromosome of *B. pertussis*. Thus, a genetically detoxified pertussis toxin molecule has been produced using the new mutant strain of bacteria. This genetically modified holotoxin should have the same immunogenic properties as native pertussis toxin and it can be used as a vaccine antigen without chemical inactivation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00480-04 LPB

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Sr. Staff Fellow	LPB, NIAID
OTHERS:	W. J. Simpson	Visiting Associate	LPB, NIAID
	R. E. Thomas	IRTA Fellow	LPB, NIAID
	R. H. Karstens	Bio. Lab. Tech. (Micro)	LPB, NIAID

COOPERATING UNITS *(if any)*

Center for Disease Control, Fort Collins, CO (A. M. Barnes, T. Quan); Stanford University, CA (K. McDonough and S. Falkow)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to develop molecular approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States. The first objective was to develop a sensitive DNA hybridization probe to specifically identify *Yersinia pestis*, the causative agent of bubonic plague, in experimentally infected fleas. This was achieved in collaboration with K. McDonough and S. Falkow at Stanford University. *Y. pestis* infection in individual fleas of three species (*Xenopsylla cheopis*, *Thrassis bacchi*, and *Diamanus montanus*) were detectable using a 1,000 base pair restriction fragment cloned from the pesticin plasmid. The same probe also detected *Y. pestis* in impression smears of spleen and liver of experimentally infected mice. Primers for use in a polymerase chain reaction (PCR) have been constructed and will be used to detect *Y. pestis* in experimentally infected fleas. Protocols for the purification of murine toxin and Fraction 1 antigen of *Y. pestis* are being developed to provide reagents for alternative methods for the rapid detection of *Y. pestis* in infected fleas. The gene for Fraction 1 has been cloned in *Escherichia coli* using the λZAPII expression vector. The recombinant *E. coli* expresses significant amounts of the F1 antigen and protects mice that are challenged with virulent *Y. pestis*. We will evaluate the feasibility of this in developing a subunit vaccine for plague based on the F1 antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00488-03 LPB

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructural Analysis of Antigenic Determinants in Pathogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LPB, NIAID
OTHERS:	S. F. Hayes	Bio. Lab. Tech. (Micro)	LPB, NIAID
	D. Corwin	Bio. Lab. Tech. (Micro)	LPB, NIAID
	W. Burgdorfer	Scientist Emeritus	LPB, NIAID
	D. W. Dorward	Staff Fellow	LPB, NIAID
	L. L. Lubke	Biologist	LPB, NIAID
	T. G. Schwan	Sr. Staff Fellow	LPB, NIAID

COOPERATING UNITS *(if any)*

LPVD, RML, NIAID; LMSF, RML, NIAID
Dept. of Medical Microbiology - Stanford University School of Medicine
Dept. of Microbiology and Immunology - University Rochester Medical Center

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.13	0.4	1.73

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project serves to consolidate and to expand our efforts at identifying, localizing, and characterizing genes and gene products which are important in host pathogen relationships. While we have used colloidal gold and ferritin labeling to identify important cellular components which are located on the surface of various pathogens, new techniques of cryofixation, cryoultramicrotomy and immuno-labeling will allow precise localization of important antigens which may be located inside the cell. A broad range of molecular techniques will allow tracking of antigens through synthesis, processing and genetic control modulation. Recently, intimate associations between nucleic acids and elaborated membrane vesicles in cultures of *Borrelia burgdorferi* and *Neisseria gonorrhoeae* were described. In both species, evidence indicated that DNA was packaged and exported within these structures, termed blebs, and that a proportion of the DNA was protected from exhaustive digestion with pancreatic DNase I. In *N. gonorrhoeae* bleb preparations, DNAase-protected R-plasmids specifying penicillinase production were efficiently incorporated and expressed by wild-type recipient gonococci, suggesting bleb-mediated transfer of DNA can occur, perhaps constituting a previously undocumented genetic exchange mechanism. Subsequent experiments identified DNA-binding proteins specifically expressed in two unique fractions of gonococcal blebs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00489-03 LPB

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification and Biochemistry of Molecularly Cloned *Bordetella* Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jerry M. Keith	Head	LPB, NIAID
OTHERS:	K. Vadheim & S. Strachan	Guest Workers	LPB, NIAID
	M. Szulczynski & Y. Lobet	Visiting Fellows	LPB, NIAID
	K. Kaljot	NRSA Researcher	LPB, NIAID
	S. Northcutt & S. Smith	Microbiologists	LPB, NIAID
	D. Brown	Sr. Staff Fellow	LPB, NIAID
	W. Cieplak	NRC Associate	LPB, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.55	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pertussis toxin (PTX) is an oligomeric protein produced by *Bordetella pertussis*, the etiologic agent of whooping cough or pertussis. PTX exerts a diverse array of biological effects both *in vivo* and *in vitro* and is thought to occupy a central role in the pathogenesis of pertussis. Importantly, PTX has been demonstrated to be an important component of acellular vaccines. PTX appears to exert many, if not most, of its pathobiological effects through the NAD-dependent ADP-ribosylation of guanine nucleotide-binding regulatory proteins of the membrane-bound adenylate cyclase complex of mammalian cells; therefore, PTX belongs to the group of ADP-ribosylating bacterial protein toxins that include cholera toxin, the *Escherichia coli* heat-labile toxins, diphtheria toxin and *Pseudomonas* exotoxin A. Development of new and safer whooping cough vaccines is a major priority of health policy organizations and vaccine manufacturers, Evidence accumulating from clinical trials of acellular pertussis products strongly indicates that pertussis toxin will be a necessary, and perhaps sufficient, component of any new vaccine. Anticipating the need for purer and non-reactogenic reagents, we have cloned and recombinantly expressed the genes for each of the toxin component proteins in *Escherichia coli*. This project involves purification and characterization of these protein antigens produced by the host system. In many instances, these expressed proteins represent up to 40% of the total cell protein; however, these proteins are often insoluble and form inclusion bodies in the *E. coli* cell. Various techniques were developed to solubilize these proteins. We have succeeded in purifying various versions of subunits S1, S2, and S4. Manipulation of the gene segment encoding the A protomer (S1 subunit) has permitted identification of regions possessing both immunodominant and immunoprotective epitopes as well as sites critical for enzymatic activities. We expect that our recombinant reagents will allow us to develop a stable, safe, and efficacious acellular vaccine against pertussis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00490-03 LPB

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Protection Studies Using Synthetic and Recombinant Pertussis Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jerry M. Keith	Head	LPB, NIAID
OTHERS:	K. Kaljot	NRSA Researcher	LPB, NIAID
	S. Northcutt	Microbiologist	LPB, NIAID
	W. Cieplak	NRC Associate	LPB, NIAID
	S. Strachan	Guest Worker	LPB, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.15	0.7	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves performing studies directed towards the development of vaccines using recombinant DNA technology to synthesize immunogenic peptides from microbial agents. The objective is to identify specific natural or genetically modified proteins which when used as immunogens will protect against challenge by *Bordetella pertussis*, the causative agent of whooping cough. These antigens will then be considered for use in a new generation consisting of five subunits, which is a major protective immunogen in whooping cough vaccines. Our initial effort involves evaluating individual subunits of pertussis toxin produced synthetically using recombinant DNA technology. Our earliest results suggested that the standard mouse lethal intracerebral challenge (ic) model was incapable of reliably identifying protective pertussis components and are variably reliable in demonstrating protection with a commercial available whole cell vaccine. By modifying the ic challenge to include a small dose of active pertussis toxin, which is incapable of stimulating immunoprotection following immunization with both purified components and recombinant component subunits. However, mice immunized with the recombinant subunits with added subimmunogenic doses of pertussis toxin were unable to mount a response that protected them from the pathophysiologic effects of the toxin. The results suggest that anti-subunit antibodies may play roles in preventing disease other than those involving the neutralization of pertussis toxin activity

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00491-03 LPB

PERIOD COVERED

October 1, 1988, to January 1, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Antibodies of IgE Isotype in Immunologically Medicated Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John J. Munoz	Research Microbiologist	LPB, NIAID
OTHERS: M. G. Peacock	Microbiologist	LPB, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Immunopathology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of antibodies in delayed-type hypersensitivity (DTH) and autoimmune reactions has not been clearly shown. However, vasoactive substances and capillary permeability changes have been shown to play a role. The most important cell involved in the release of vasoactive substances in the vicinity of capillaries is the mast cell. These cells are degranulated by specific antigens reacting with the IgE class of immunoglobulins bound to their Fc epsilon receptors. Degranulation of mast cells is also mediated, although less efficiently, by antibodies of the IgG_1 class.

We have shown that Ptx increases the number of lymphocytes containing IgE on their surfaces and that this increase is mainly due to an increase in the number of lymphocytes with Fc epsilon receptors. We have also shown that hyperimmune antisera to *Bordetella pertussis* can be depleted of IgG and still retain their ability to protect mice from IC challenge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00492-03 LPB

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Sr. Staff Fellow	LPB, NIAID
OTHERS:	W. J. Simpson	Visiting Associate	LPB, NIAID
	W. Burgdorfer	Scientist Emeritus	LPB, NIAID
	S. Czub	Visiting Fellow	LPB, NIAID
	M. E. Schrumpf	Bio. Lab. Tech. (Micro)	LPB, NIAID
	R. H. Karstens	Bio. Lab. Tech. (Micro)	LPB, NIAID

COOPERATING UNITS *(if any)*

Southampton Hospital, Southampton, NY (A. MacDonald).

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.4	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to (1) determine if genes present on plasmid DNA of *Borrelia burgdorferi*, the causative agent of Lyme disease, control infectivity, (2) characterize these plasmids, (3) clone the infectivity genes and express those components that promote borrelia infection in mammals, and (4) determine immunogenic properties of components responsible for infection. The reduction in the number of detectable plasmids with the loss of infectivity suggests that gene(s), encoding for components related to infectivity, may be present on one or more of these extrachromosomal elements. A 7.6 kilobase (kb) pair circular plasmid was first identified as a strong candidate for regulating infection. Numerous fresh strains of *B. burgdorferi* were examined for their infectivity and presence of the 7.6 kb circular plasmid. While most strains that were infectious in Syrian hamsters had the 7.6 kb plasmid, two strains that were infectious lacked this plasmid or sequences similar to it elsewhere in their genomes. We are now cloning restriction fragments from the 22 kb linear plasmid which was also lost at the same time that strain Sh-2-82 became noninfectious, to examine the role of this plasmid in infectivity. Investigations concerning *in vivo* antigenic changes in white-footed mice, the natural reservoir for *B. burgdorferi*, have demonstrated that antigenic variation during infection is significant and may play an important role in the spirochete's ability to maintain persistent infections in spite of a pronounced humoral immune response.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00552-01 LPB

PERIOD COVERED

January 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathobiology of Bacterial Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Witold Cieplak, Jr.	Senior Staff Fellow	LPB, NIAID
OTHERS:		
C. Cluff	IRTA Fellow	LPB, NIAID
S. G. Smith	Microbiologist	LPB, NIAID
M. G. Peacock	Microbiologist	LPB, NIAID
Y. Lobet	Visiting Fellow	LPB, NIAID

COOPERATING UNITS *(if any)*

National Institute Environmental Health Sciences (M. Rodbell), Research Park Triangle, NC

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.57	.97	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bacterial protein toxins, such as pertussis toxin and cholera toxin, are known to exhibit significant effects on the immune system. Pertussis toxin is produced by *Bordetella pertussis* and catalyzes the ADP-ribosylation of guanine nucleotide-binding proteins. Pertussis toxin exerts mitogenic/activating effects on lymphocytes and is a potent stimulator of IgE synthesis in rodents; the latter activity may be important in the development of human immediate-type hypersensitivity states after pertussis immunization. To more completely understand the molecular basis and importance of these immunological effects, we have recently initiated studies designed to examine the role of the IgE potentiation in the vaccinogenic potential of pertussis toxin and the role of ADP-ribosyltransferase activity in eliciting such immunological perturbations. These studies involve assessing the protective immunogenic activity of pertussis toxin in mutant strains of mice that are deficient in the ability to synthesize IgE. In other studies, mutant forms of pertussis toxin that have severely impaired ADP-ribosyltransferase activity have been determined to have a recently described and less obvious enzymatic activity that may be significant to their potential use as vaccine constituents. The influence of these latter activities on the toxic and activating properties of pertussis toxin is being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00553-01 LPB

PERIOD COVERED

January 1, 1989, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology and Immunology of Pathogenic *Campylobacter* spp.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Witold Cieplak, Jr.	Senior Staff Fellow	LPB, NIAID
OTHERS:		
Christopher Cluff	IRTA Fellow	LPB, NIAID
Marius G. Peacock	Microbiologist	LPB, NIAID

COOPERATING UNITS *(if any)*

VA Medical Center (M. Blaser), Denver, CO

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.75	.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Members of the genus *Campylobacter*, particularly *C. jejuni* and *C. coli*, are significant causes of bacterial enteritis in both developed and underdeveloped countries. Studies have been recently initiated that will investigate the important virulence determinants associated with these organisms and the manner in which they interact with the host to produce disease. To these ends we are developing an array of polyclonal and monoclonal antibodies to define surface and other structures that might be related to virulence and pathogenesis. A genomic library of pathogenic *C. jejuni* is also under construction, and attempts are being made to identify potential enterotoxin genes using oligonucleotide probes designed from conserved sequences found in potentially related toxins produced by other bacterial species. Studies that are designed to identify components that are involved in binding to and invading human epithelial cells are also in progress. Finally, using cross-reactive antisera, we have identified a member of a family of highly-conserved heat-shock (stress response) proteins that are present in *C. jejuni*; we are investigating the role of this protein in intracellular survival and its role as a potentially important antigen in the immune response to the organism. We anticipate that these studies will lead to insights concerning the pathogenesis of *Campylobacter*-caused disease, and may provide useful information that may be applied to development of preventative and/or therapeutic modalities for these and other enteric disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00554-01 LPB

PERIOD COVERED

October 1, 1988, to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Characterization of Microbial Genes and Nucleic Acid Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Claude F. Garon	Chief	LPB, NIAID
OTHERS:		
D. W. Dorward	Staff Fellow	LPB, NIAID
T. G. Schwan	Sr. Staff Fellow	LPB, NIAID
L. L. Lubke	Biologist	LPB, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.23	0.4	0.83

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lyme borreliosis is now the most common arthropod-borne disease in the United States. *Borrelia burgdorferi*, the causative agent, has been isolated from humans, mammals, birds and arthropods and is cultivable in the laboratory. Careful structural analysis of the DNA content of several early passage isolates has revealed, in addition to a 900 kb linear genome, a unique mixture of terminally crosslinked linear and covalently-closed, circular DNA molecules ranging in size from a few to 50 kilobases in length. Often DNA patterns appear to differ among isolates and to vary during laboratory passage. Given the number of molecules in this apparent extrachromosomal pool, the presence of genes for major surface proteins located on relatively small linear DNA molecules, the uniqueness of the DNA among isolates, and the structural features of these molecules, we have asked if this pool of molecules might not function as a collection of minichromosomes. This view has been supported recently by the description of a similarly segmented arrangement of DNA molecules in *B. duttonii* - an agent of relapsing fever.

LPVD

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1989 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1988 to September 30, 1989

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

Dr. Dahn Clemens, IRTA, from Colorado State University, Fort Collins, Colorado, working with Dr. Marshall Bloom.

Dr. Michael Robertson, Medical Staff Fellow, from Seattle, Washington, working with Dr. Bruce Chesebro.

Dr. Richard Marsh, Special Volunteer, from the University of Wisconsin, Madison, Wisconsin, working with Dr. Richard Race.

Dr. Gerald Spangrude, Special Volunteer, from the Walter and Eliza Hall Institute of Medical Research, Victoria, Australia, working with Dr. Bruce Chesebro.

Departures:

Dr. Richard Buller, Staff Fellow, taking a position in the Department of Pediatrics, St. Louis Childrens Hospital, St. Louis, Missouri.

Dr. Chiaki Ishihara, Guest Researcher, returning to work in the Laboratory of Animal Experiment, Institute of Immunological Science, Hokkaido University, Sapporo, Japan.

Dr. Ditte Tornehave, Visiting Associate, taking a position in the Institute for Anatomy and Cytology, University of Odense, Odense, Denmark.

Summer students were David Stokesberry from Oklahoma State University, Stillwater, OK; Bradley Berry from Caroll College, Helena, MT; and Kristi Hurley from College of St. Catharine, St. Paul, MN.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1988 to September 30, 1989

LPVD - Significant Accomplishments - 1989

1. Several host genes have been identified which are involved in resistance to induction of degenerative brain disease by the scrapie agent.

2. Altered cellular proteins have been found in mouse brain tumor cells infected *in vitro* with the scrapie agent. These proteins could provide clues regarding the nature and action of this agent.

3. The number of AIDS patient peripheral blood cells producing infectious HIV has been directly measured using a sensitive infectivity assay developed at LPVD. The assay can detect as little as 1 HIV-positive cell out of 1 million cells. AIDS patients were found to vary considerably in the number of positive cells detected.

4. Antibodies to the human major transplantation antigens (DR) were found to partially inhibit HIV infection. This suggests a possible role for these antigens in virus spread.

5. New retroviral gene sequences involved in causing anemia by the killing of red blood cell precursors have been identified in a mouse retrovirus system.

6. A mouse gene has been found to control the immune response to the Friend retroviral envelope protein. This same gene determined the success or failure of induction of protective immunity to this virus following vaccination with recombinant vaccinia virus expressing Friend envelope protein.

7. A recombinant retrovirus has been constructed which can induce rapid (18-20 days) severe degenerative brain and spinal cord disease in mice. The analysis of the different genetic components of this virus should give information regarding biological mechanisms of disease induction.

8. A retroviral envelope gene has been found to be expressed on many blood precursor cells found in bone marrow, thymus, spleen, and fetal liver. This envelope gene has now been partially cloned and detailed DNA sequence and antigenic analyses are in progress.

9. New recombinant retroviruses have been found which might provide information as to the events involved in generation of polytropic leukemia-inducing retroviruses in mice.

10. Neutralization of Aleutian disease virus by antiviral antibody has been demonstrated *in vitro.* This finding suggests that vaccination against this disease might be possible.

11. An infectious molecular clone of Aleutian disease virus, strain ADV-G, has been isolated. This clone should prove useful in studies of pathogenesis and immune protection.

12. Cytotoxic T lymphocytes capable of killing rabies virus-infected cells have been generated from spleens of mice with rabies infection. The role of these cells in resistance to rabies is being studied.

13. Rabies virus infection has been found to induce alterations in the types of lymphocytes found in thymus, spleen and lymph nodes of mice. These effects might influence resistance to disease.

14. The role of estrogens in down regulation of hamster serum female protein is being studied and compared to the role of these hormones in inducing liver tumors in certain hamster strains.

LPVD - New Initiatives - 1989

1. Effect of different cell types on induction of variation in lentivirus genes during infection.
2. Role of mixed retroviral infections in enabling HIV to infect new cell types by providing new viral envelope proteins.
3. Use of envelope genes from retroviruses which infect brain cells to construct cell lines capable of delivering foreign genes to brain cells.
4. Analysis of retroviral core proteins as protective immunogens in recombinant vaccinia virus vaccines.
5. Study of physical properties of scrapie agent from laboratory cultured brain tumor cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00072-18 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Lodmell	Scientist Director	LPVD, NIAID
Other:	Masami Sugamata	Visiting Scientist	LPVD, NIAID
	Linda L. Perry	Expert	LPVD, NIAID
	Joseph J. Esposito		CDC
	William J. Bellini		CDC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal objective of these studies is to determine host and viral factors that influence the genetically-controlled resistance of inbred mouse strains to street rabies virus (SRV). Presently we are investigating whether cytotoxic T cells (CTLs) correlate with resistance to infection, as well as the clinical course of disease.

Because we are studying inbred strains of mice with different H-2 haplotypes, and varying susceptibilities to rabies virus, H-2 restricted CTL assays were developed to use with A/WySn (H-2^a), BALB/cByJ (H-2^d), SJL/J (H-2^s), and A.SW/SnJ (H-2^s) strains of mice. Conventional nonadherent target cells were used for assays with the H-2^a and H-2^d mice, whereas the H-2^s target cells were monolayers of SV-40 transformed fibroblasts. All targets proved to be highly sensitive to rabies virus specific CTL lysis in that memory CTLs were readily demonstrated in all mouse strains following *in vitro* amplification with rabies virus-infected stimulator cells. We were unable to detect primary CTLs in spleens of mice inoculated intraperitoneally (ip), intramuscularly, intracranially or subcutaneously (sc) with virulent SRV or the less virulent ERA rabies virus. CTLs were characterized as H-2 restricted, Thy-1.2^+, Lyt 2.2^+, Lyt-1.2^- and not to posses NK activity. CTLs were never elicited in any of the mouse strains following inoculation with the identical concentration of the 10% mouse brain suspension of SRV that had previously established mouse strain susceptibility differences. Tissue culture preparations of ERA or SRV that either killed or did not kill BALB/cByJ mice, depending on the route of inoculation, readily elicited CTLs. SRV resistant and susceptible mice produced similar CTL responses to these viruses.

SRV susceptible A/WySn mice were vaccinated via tailscratch with $1X10^6$ PFU of recombinant raccoonpox virus vaccines expressing rabies virus glycoprotein (GP) or nucleoprotein (NP) under $P_{7.5}$ (early-late class) or P_{11} (late class) promoters. Rabies virus specific CTLs and neutralizing antibody responses were determined following vaccination or vaccination and subsequent SRV challenge. 100% of the mice receiving vaccines expressing GP under early/late or late promoters survived following sc or ip challenge with high concentrations of SRV. In contrast, mice vaccinated with recombinants expressing NP did not survive. CTLs and neutralizing antibodies were induced only by recombinants expressing GP. Following ip-inoculation, recombinant Wyeth and Copenhagen strains of vaccinia virus expressing rabies NP also were ineffective in protecting A/WySnJ mice against high concentrations of sc-inoculated SRV. We are continuing to investigate the importance of CTLs and antibody in resistance/recovery to SRV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00074-17 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	M. Miyazawa	Visiting Associate	LPVD, NIAID
	M. Robertson	Medical Staff Fellow	LPVD, NIAID
	C. Ishihara	Special Volunteer	LPVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	3.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main aim of this work is to define genetically controlled mechanisms of host defense against retroviral diseases. The virus being studied is the Friend virus complex (FV) which induces rapid erythroleukemia in both newborn and immunocompetent adult mice. Results to date have defined 3 mouse genes which are all important for spontaneous recovery from FV leukemia. These are H-2I-A, H-2D and Rfv-3, which is not H-2 linked. Rfv-3 controls development of a humoral anti-viral antibody response even in the presence of ongoing infection, and is necessary, but not sufficient, for occurrence of spontaneous recovery. H-2I-A has now been shown to control an immune response gene for the Friend virus envelope protein. H-2D is correlated with a strong cytotoxic T lymphocyte response to viral antigens and appears to be most important for spontaneous recovery. Thus, the recovery process appears to involve a combination of genes influencing several humoral and cellular immune mechanisms.

Studies of protective immunity have been done using killed virus, purified viral proteins, and genes for individual viral proteins expressed in recombinant vaccinia viruses. The results indicate that the envelope protein is the strongest protective immunogen; however, its success can be dependent on the presence of the appropriate H-2I-A-linked immune response gene. However, lack of this gene can be overcome by using appropriate strong adjuvants or high amounts of antigen to immunize. More recent experiments indicate that vaccinia expressing the Friend virus core polyprotein can protectively immunize some mouse strains, but this effect is weaker than immunization with vaccinia expressing the Friend virus envelope protein. Future experiments are aimed at creating deletions in both *env* and *gag* genes to define the most important antigenic regions for immunogenicity and T cell recognition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00085-12 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	S. Alexandersen	Asst. Professor	Royal Vet. and Ag. U., Vulowsvej, Denmark
	S. Mori	Visiting Fellow	LPVD, NIAID
	Wu Wei	Special Volunteer	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 2.7	PROFESSIONAL: 2.0	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The scope of this project is to elucidate pathogenic mechanisms involved in infections of mink with Aleutian mink disease parvovirus (ADV).

In the past year, we have developed the methodology to perform in situ hybridization (ISH) in conjunction with immunohistochemical staining (IMHI) for protein antigens. This combined method has been used to identify ADV nucleic acids and proteins in the same infected cell during a permissive infection.

A series of mouse monoclonal antibodies that recognize mink immunocyte antigens has been prepared and characterized. It is now possible to discriminate several discreet populations of mink immunocytes (equivalent s of CD5, CD8 and class II MHC antigens) by flow cytometry and IMHI. Using these reagents, 2 forms of ADV induced nephritis lesions have been identified. One form is a typical immune complex nephritis complete with glomerular deposition of immune complexes, but the other is an interstitial nephritis characterized by the infiltration of CD8 lymphocytes.

In vitro neutralization of ADV-G has been demonstrated. Sera from ADV infected mink, heterologous anti-capsid antibody and several anti-ADV monoclonal antibodies can neutralize infectivity of ADV-G in cell culture if virus is pretreated with ether. Infected mink develop high titers of neutralizing antibody after infection with ADV, but still succumb to Aleutian disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00086-12 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
Other:	Markus Czub	Visiting Fellow	LPVD, NIAID
	William Lynch	IRTA	LPVD, NIAID
	Stefanie Czub	Visiting Fellow	LPB, NIAID
	Bruce Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is focussing on the mechanisms involved in the pathogenesis of retrovirus induced neurodegeneration. We are studying a paralytic disease induced by a retrovirus originally isolated from wild mice. The virus causes both leukemia and CNS degeneration both diseases in the wild having long incubation periods of from 6-12 months. We are interested in several aspects of this disease including: 1) The viral and host determinants which control the incubation period; 2) the viral and host factors which influence the replication of the virus in the CNS; 3) the nature of viral gene expression in the CNS; 4) features of viral expression and or replication which determine neurovirulence. Through the construction of chimeric viruses we have found that the tempo and level of infection of the CNS are multigenic effects being influence by env, LTR as well as sequences within the gag-pol region of the viral genome. We have identified a viral genomic motif containing LTR gag-pol sequences from a strain of F-MuLV which facilitates virus replication within the CNS, and into which we have introduced a variety of env genes. This system has allowed us to evaluate the relative neurovirulence potentials of env genes from a variety of murine retroviruses and should prove useful for defining the env sequences which influence neurovirulence. We have constructed a chimeric virus which induces neurodegenerative disease in mice in 18-20 days with remarkable stereotypy. 100% of the mice develop disease and the pathology is identical to that cause by field isolates (ie spongiform degeneration of grey matter). This system has provided a means of studying the early events in the pathogenesis of this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00260-08 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	R. Buller	Staff Fellow	LPVD, NIAID
	J. Portis	Medical Officer	LPVD, NIAID
	L. Evans	Chemist	LPVD, NIAID

COOPERATING UNITS *(if any)*

Dr. M. Sitbon, Hopital Cochin, Paris, France

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is focused on study of mechanisms of pathogenesis of murine leukemia viruses. Most recent experiments have involved a polytropic recombinant Friend mink cell focus inducing virus (F-MCF 98D) which causes both leukemia and central nervous system disease in mice. Host genetic resistance to the CNS disease has been observed to involve recovery from active virus infection and was found to be controlled by two independent genetic loci. One of these genes was linked to a gene encoding an endogenous retroviral envelope expressed on early hemopoietic cells and might act by a viral interference mechanism. The linkage of another gene was not determined, but its presence appeared to be associated with development of antiviral immune response. Future efforts are aimed at molecular cloning of this unusual neurotropic retrovirus in order to analyze various gene sequences for their contribution to the pathogenesis observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00262-08 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Coe	Medical Officer	LPVD, NIAID
Other:	D. Tornehave	Visiting Associate	U of Odense, Denmark
	Ray Monk	Sr. Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

See attached page

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The serum of Syrian hamsters contains a protein which is called female protein (FP) because it is preferentially expressed in female hamsters with serum levels 100-500 fold greater than male hamsters. FP is a pentraxin, a family of proteins commonly expressed in vertebrates as two proteins which are homologs to human C-reactive protein (CRP) or serum amyloid P component (SAP). Hamster FP is an interesting model because only in hamster is the control of the hepatic synthesis of pentraxin regulated by sex steroid. The genetic mechanism responsible for FP regulation is of great interest and information about this novel system of steroid control and FP function may shed light on the basic physiological role of pentraxins in general. Because we have found steroid control of pentraxin synthesis is quite different among various hamster species, the molecular basis for differential expression is being evaluated. Of particular interest is the finding that synthesis of FP in Armenian hamster is down regulated by exogenous estrogen administration (in direct contrast to Syrian hamster). Exogenous estrogens are also hepatotoxic in Armenian hamster in contrast to Syrian hamster. Indeed, chronic estrogen treatment will produce hepatomas in the majority of Armenian hamsters. This remarkable susceptibility to estrogens is unique when compared to other hamsters and other vertebrates in which estrogens will promote hepatoma development only when an additional carcinoma inducing agent is used, and exogenous estrogens are generally non-cancerous when administered by themselves.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00263-08 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	Soren Alexandersen	Asst. Professor	Royal Vet. and Ag. U., Vulowsvej, Denmark
	Wu Wei	Special Volunteer	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.8	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study various aspects of genome structure and function of the Aleutian mink disease parvovirus (ADV).

In the past year we have produced a full-length molecular clone of the ADV-G strain of ADV (designated XV-B-4) and characterized its ability to initiate infections in vitro and in vivo. When Crandell feline kidney (CRFK) cells were transfected with XV-B-4, infectious ADV was generated in a permissive infection. The XV-B-4 derived virus induced ADV specific proteins and DNA intermediates in CRFK cells indistinguishable from the parent ADV-G. Adult Aleutian mink inoculated with XV-B-4 responded as though they had been infected with ADV-G, i.e., they remained asymptomatic, developed only very low levels of anti-ADV antibodies and no hypergammaglobulinemia and had no pathologic signs of Aleutian disease (AD) at necropsy. Thus, the infectious molecular clone retains the pathogenicity characteristics of the parent strain.

A series of molecular clones expressing segments of the right or capsid gene open reading frame (ORF) in bacteria was developed. These prokaryotic expression clones were used in Western blots to map the epitopes recognized by 2 monoclonal antibodies (mAB Y-2-9 and mAB 47) and a large panel of ADV-infected mink sera. The reactivity pattern suggested that Y-2-9 recognized an epitope in the 30% of the right ORF closest to the amino terminus and that 47 recognized an epitope located in the carboxy 30%. The reactivity of antibodies from infected animals suggested that mink recognized epitopes found in all segments of the right ORF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00264-08 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Endogenous Retroviruses Expressed During Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
Other:	R. Buller	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID
	M. Miyazawa	Visiting Scientist	LPVD, NIAID

COOPERATING UNITS *(if any)*

Dr. Gary Van Zant, Dept. Molec. and Cell Biology, Texas Tech Univ., Lubbock, TX.

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously identified a retroviral env gene which is polymorphic among various mouse strains, having at least two alleles one of which is polytropic-like and one of which is xenotropic like (distinguished by monoclonal antibodies). The polytropic gp70 is expressed by mouse strains which are resistant to the induction of erythroleukemia induced by neonatal inoculation of F-MuLV and genetic analysis has shown that this resistance is in part due to the presence of this gene. We have found that the gp70 proteins encoded by these genes are expressed in normal mice by a population of cells which make up the pool of erythroid, granulocyte/monocyte and T-lymphocyte progenitors. We have extended these observations to the second allele of this gene, that which encodes a gp70 resembling that of xenotropic viruses. Genetic analysis also revealed that this gp70 functions as one of several resistance genes, which together impart resistance to erythroleukemia. The mechanism by which both of these alleles impart resistance to leukemogenesis involves restricting expression of recombinant polytropic viruses which appear to be the proximal agents in the induction of erythroleukemia. Viral interference has been proposed as the mechanism responsible for this form of genetic resistance to retroviral disease. We have tested this hypothesis in vitro and found that classical viral interference patterns are not induced by this env gene. We have now cloned one of these alleles (xenotropic-like) from a C-DNA library prepared from mouse 3T3 cell line derived in this laboratory. This sequence consists of approximately the 3' two thirds of an env gene plus the entire 3' LTR. Sequence comparison reveals a high level of homology with endogenous polytropic proviral sequences previously cloned from genomic DNA. This sequence has been introduced into a vaccinia expression system and the chimeric env protein will be analyzed to determine whether it reacts with the appropriate monoclonal antibodies. We have yet, however, to identify the 5' regulatory sequences which control expression of this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00265-08 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Other:	B. Caughey	Staff Fellow	LPVD, NIAID
	K. Neary	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. M. Buchmeier, Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Scrapie is a spongiform encephalopathy of sheep and goats. Similar diseases have been described in mink, mule deer, elk, and most recently, cattle. At least three human diseases, Creutzfeldt-Jakob disease, Kuru, and Gerstmann-Sträussler syndrome, are histologically indistinguishable from scrapie. Available evidence suggests that all of these diseases are caused by similar transmissible agents which are particularly interesting because they may not depend upon nucleic acid in order to replicate. Interestingly, preparations that contain large amounts of scrapie infectivity contain an aggregated proteinase K (PK)-resistant form of an endogenous protein, PrP. Experimental evidence suggests that PrP is important in disease pathogenesis. However, considerable controversy surrounds the relationship between PrP and the infectious agent of scrapie. A few investigators believe the PK-resistant form of the protein is the scrapie agent. Alternatively, it's possible that the protein could be a component of the agent or merely accumulate as a byproduct of disease.

We have developed an *in vitro* scrapie infected cell culture system which we are using to study the infectious agent and PrP. We have developed procedures for detecting both the normal (PK-sensitive) and PK-resistant forms of PrP. The PK-resistant form was detected in scrapie infected but not in uninfected cultures. Experiments are underway to determine if a reliable quantitative relationship can be shown between the detection of PK resistant PrP and infectivity. If such a relationship exists, the length of time required to analyze agent in unknown samples could be reduced from eight months to one week. The biochemical basis for the differences between the normal and scrapie-modified (PK-resistant) PrP forms is not known. Thus, we are seeking ways to discriminate the two forms of PrP which might suggest differences in post-translational modification and/or subcellular location. To date we have determined that the forms of PrP differ in both their aggregation state and sensitivity to release from intact cells by proteases and a phospholipase. To determine the function of PrP in scrapie infectivity, we are also isolating mouse neuroblastoma cells which express greatly reduced levels of PrP. We will examine these cells for their ability to harbor scrapie infectivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00266-08 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. H. Evans	Chemist	LPVD, NIAID
Other:			

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic variation resulting from recombination and point mutation is well documented in retroviruses. Murine leukemia viruses (MuLVs) readily undergo recombination with endogenous retroviral sequences to generate host range varients which, in many cases, have been directly implicated in leukemogenesis. Among lentiviruses such as the equine infectious anemia virus (EIAV) and the human immunodeficiency virus (HIV), point mutation has been postulated as a mechanism leading to variants which excape a host immune response or which possess an altered tissue specificity and pathogenicity. The major emphasis of this project has been genetic alteration as a result of recombination in murine retroviruses. Oncogenic recombinant viruses arise spontaneously in high leukemia incidence mouse strains through a complex mechanism involving multiple recombination steps which are not well understood. Our previous studies in high leukemic mouse strains identified a new MuLV species in very young mice which led to a detailed model for the generation of oncogenic MuLVs. In vitro co-cultivation of MuLVs corresponding to putative intermediates in the model resulted in the spontaneous generation of a recombinant MuLV with an altered host range and cytopathic properties quite similar to oncogenic MuLVs which arise in vivo. We have determined that the recombinant is not oncogenic in vivo and structural analysis of its genome suggests requirements for oncogenicity which have not been previously recognized. We have recently broadened the scope of the project to develop procedures to precisely measure the rate of point mutation of MuLVs with the aim of applying such procedures to lentiviruses. Initial determination of the point mutation rate of an MuLV through a single replicative cycle indicates a rate approximately 10-fold lower than published estimates. During the course of these studies we have developed a monoclonal antibody which is reactive with a nearly ubiquitious epitope located near the carboxy termini of the envelope glycoproteins of MuLVs. The antibody is effective in a variety of immunological procedures and possesses neutralizing activity to many MuLVs including representative viruses of all known interference groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00418-06 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	S. Carpenter	Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves studies of the horse lentivirus, equine infectious anemia virus (EIAV). This virus is structurally and serologically related to the human AIDS virus, HIV, and provides a useful model for comparative studies. Experiments so far have characterized viral variants isolated serially during infection to determine the possible role of the antiviral immune response in selection of variants. Results indicate variants are not selected by resistance to neutralizing antibody, but may be selected at the level of virus-infected cells. More recently, viral isolates were analyzed before and during *in vitro* cultivation in various cell types, and selection of variants with tropism for particular cell types was documented. This type of variant selection *in vitro* appears to be important for the loss of ability of *in vitro* cultivated EIAV to cause rapid clinical disease in horses.

Current experiments include attempts to isolate molecular clones of several EIAV isolates. These will be used to analyze viral gene structure and function and to express viral proteins in immunogenic vector systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00468-04 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Human AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	None		

COOPERATING UNITS *(if any)*

Julie Metcalf, NIAID, Bethesda, MD; Diane Griffin, Johns Hopkins University School of Medicine, Baltimore, MD.

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.4	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project has been development of a sensitive quantitative assay for infectivity of human immunodeficiency virus (HIV). The assay involves use of human HeLa cells expressing the CD4 gene as targets for HIV infection *in vitro*. Foci of infection are detected by reactivity with anti-HIV polyclonal or monoclonal antibodies. The assay can measure both cell-free HIV and individual lymphocytes or other cells releasing infectious HIV. As little as 1 positive lymphocyte per million has been successfully detected. More recent studies have involved testing HIV infection in human astroglial or squamous cell carcinoma cells expressing the CD4 gene. The results indicate that CD4 is not the only gene product required for efficient productive infection of human cells. Future studies will focus on identifying other elements required for successful HIV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00524-02 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Factors in Susceptibility and Resistance to Rabies Virus in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	D. Lodmell	Staff Scientist	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunogenetic mechanisms of susceptibility or resistance to street rabies virus (SRV) are being investigated in a murine model system. Previous observations of involution of the primary lymphoid organs and massive lymphoid depletion over the 12 to 14 day course of disease in susceptible A.SW mice have been shown to be due to disease-induced dysfunctions in adrenal gland activity. Thus, adrenalectomized A.SW mice do not exhibit involution or cellular depletion of the lymphoid system. The mechanism of adrenal dysfunction in this disease may be attributed either to SRV infection of the adenohypophysis with subsequent alterations in the regulation of ACTH-induced adrenal secretion of corticosteroids, as suggested by other investigators, or due to the stress associated with feed deprivation during terminal disease, shown in my studies to stimulate similar levels of lymphoid depletion. The failure of adrenalectomized mice to mount a more vigorous humoral response to infection or display any increase in survival argues against a determining role of hormone-induced lymphoid toxicity in SRV susceptibility, however. Deficient T_h recognition of viral antigens can not be the sole primary defect in these mice since IgM as well as IgG antibody responses are diminished in susceptible versus resistant hosts. Neither does the Igh^e encoded repertoire of B cell antigen receptors appear defective for SRV since antibody responses and survival times of Igh congenic C.A-21 mice (BALB/c, Igh^e) is identical to that of the BALB/c (Igh^a) resistant parent, both strains responding significantly stronger than the A.SW or A/WySn (Igh^e) susceptible lines. Alternate explanations for the susceptible phenotype of A strain mice are now being sought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00550-01 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

EAE: Immunoregulation of Relapsing Disease and Interaction with Rabies Virus

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	None		

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Experimental allergic encephalomyelitis (EAE) is a T cell-mediated autoimmune demyelinating disease induced by immunization with myelin basic protein (MBP). In the (SJLXPL)F_1 host, post-immunization paralysis exhibits a chronic course with alternating episodes of relapse and recovery similar to that seen in multiple sclerosis, the human counterpart for this disease model. My laboratory has been investigating the immunological mechanisms regulating disease relapse in F_1 mice in studies funded by the National Multiple Sclerosis Society. We have already determined that T cell responses at all stages of disease are directed against the amino terminal peptide of MBP (a.a. 1-37) and that recovery is associated with the presence of transferrable T suppressor (Ts) activity. The influence of Ts on disease course was also demonstrated by the *in vivo* administration of mAbs known to induce or abrogate suppression, the former causing a reduced incidence and severity of clinical signs and the latter actually aggravating CNS disease signs. If suppression of MBP activity by Ts is responsible for the episodes of clinical remission, what mechanism accounts for subsequent relapse in these mice? During the past year, we have analyzed T cell responsiveness to other CNS autoantigens that may be encountered during initial T cell infiltration of the spinal cord, focusing on proteolipid protein (PLP) due to its encephalitogenic potential. Mice immunized with purified MBP do develop proliferative and DTH responses to PLP during relapse stages of disease. The magnitude of this reactivity is lower than that towards MBP, particularly in T cell proliferation assays. PLP-specific tolerization of mice did not prevent the occurrence of relapse, however, suggesting that this recognition is not critical to the chronic course of EAE.

We have also observed an interaction between EAE and SRV infection in that SRV infected SJL resistant mice consistently develop CNS disease when preimmunized with sub-encephalitogenic doses of MBP or PLP but not lysozyme. Conversely, SJL mice infected with SRV for 14 to 21 days develop limb weakness within 3 to 5 days of a subsequent suboptimal MBP immunization. Infectious virus can be recovered from the CNS of some, but not all, affected animals, rendering any mechanistic interpretation of this data premature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00551-01 LPVD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Cellular Interactions During HIV Infection *In Vitro*

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	None		

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

In vivo infection of CD4$^+$ T lymphocytes with HIV does not occur in isolation but in the context of a highly interactive network of T cells, B cells, and monocyte/macrophages. Using an *in vitro* system for primary infection of peripheral lymphocytes from normal donors, we have begun to analyze the nature of these interactions. Initial experiments have focused on the role of HLA-DR antigens in HIV infection since these glycoproteins are the natural ligands for CD4 molecules. Results indicate a 2 to 10 fold reduction in the number of HIV+ lymphocytes after 4 days of culture when anti-DR mAb is added at that time of infection. Inhibition is not due to the anti-proliferative effects of anti-DR mAbs as determined by the failure of the same mAb to suppress mitogen or lymphokine driven lymphocyte replication. The site of antibody action is still unclear, however. Anti-DR mAb did not inhibit initial HIV binding to activated lymphocytes or diminish syncytia formation. Neither do such mAbs interfere with the detection of infected cells on CD4-transfected HeLa cell monolayers, the assay system used in these studies as developed by Dr. B. Chesebro. Recent experiments suggest that anti-HLA-DR mAbs may act instead at the level of the macrophage, since antibody treatment of the population adherent to culture wells after a 3 day stimulation of peripheral lymphocytes with PHA suppressed T cell HIV infection, while similar treatment of the nonadherent T cells was without effect. Current studies are focused on the role of macrophages in HIV infection *in vitro* and their possible function as the site of anti-DR mAb mediated inhibition.

During the course of these studies, one normal blood donor was found to be less susceptible to infection by low concentrations of virus than all other donors tested. Decreased susceptibility was evident in several virus titration experiments using blood collected on three separate occasions. Expression of CD4 and HLA-DR molecules on these cells is within normal limits. We are now determining the relative sensitivity of this donor's cells to infection by wild type HIV isolates to determine the specificity of this low dose resistance.

RMOB

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1989 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1988 to September 30, 1989

Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement, and initiating payments and follow-up on financial obligations related to purchases, contracts, staff and official guest travel, and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of the power plant, and full maintenance and minor laboratory renovations in every area except electronics.

Dr. Michael Parnell came on board in early February, as the facilities veterinarian, to assume responsibility for the health and well being of all the research animals and breeding stock. Within two months after his arrival, the RML was restored to Full Accreditation by the American Association For Accreditation Of Laboratory Animal Care. Under Dr. Parnell's direction, facilities have been constructed and equipped to house and do research on a colony of 40 cynomolgus monkeys.

The physical plant was improved in a number of important aspects during the year. The contract to replace the roofs on all the buildings in the main complex was completed last fall. Two contracts were awarded in the summer; one to remove some of the asbestos in the steam distribution system and upgrade the boiler room controls and the other to upgrade the primary electrical distribution system and emergency electrical power system.

Our communications was improved last fall through the installation of a local area network (LAN), to network most of the computers within the RML as well as to connect our LAN with the other LAN's in the NIAID. Our Dimension 400 telephone system had been expanded to its full capacity and could not handle any more telephones. The problem was corrected this spring with the installation of an AT&T System 25. This is a less expensive system and allows us greater flexibility and expansion capacity.

The ability of the scientific staff to do literature searches was greatly enhanced through the acquisition of CD-Plus CD-ROM search software from Online Research for the library. This facilitates electronic searches and access to Medline from the National Library of Medicine.

The Maintenance Section has performed a number of renovation projects for the Operations Branch, LMSF and LPB in addition to their routine maintenance duties.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of over $2.3 million. Payroll is not included in this figure. It covers only the purchase of services, supplies, and equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel initiates civil service and commissioned officer personnel actions, and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office when the program is active. Also handled by Personnel are persons under the following programs: Stay-in-School, Summer Aides, Student Volunteers, Special Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in the research laboratories is prepared in a special laboratory by a technician.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Unit raises guinea pigs, 31 strains of mice, nine strains of hamsters, a colony of peromyscus and a colony of microtus. They are raising 86,900 animals annually for research from an average pool of 3,790 breeding stock (approximate figures). An additional 5,200 animals are purchased annually from outside sources, including mink, rabbits, rats, mice, hamsters and cynomolgus monkeys. After rearing, care is provided for these animals while they are under experiment. The most recent survey indicates an average daily holding of 23,820 small animals.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the contracts for Security, Custodial, operation of the Boiler Room and the Glassware Unit with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Boiler Room operation provides heat, steam, compressed air, vacuum, and emergency power to the entire laboratory complex. Glassware is cleaned and sterilized in the Glassware Unit for reuse in the laboratories.

The Maintenance Department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning, and refrigeration, including ultra-low temperature boxes. With the exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.

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40

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